ZAPOROZHYE STATE MEDICAL UNIVERSITY
THE DEPARTMENT OF INTERNAL DISEASES №3

Dotsenko S.Y., Sychov R.O., Tokarenko I.I.,
Shevchenko M.V., Chornaya I.V., Kulynych O.V.

CLINICAL IMMUNOLOGY AND ALLERGOLOGY

(tutorial for practical classes)

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Authors:
1. Dotsenko S.Y. - Head of the Department of Internal Diseases №3, Zaporozhye State Medical University, Professor, MD, PhD, Doctor of Medical Sciences
2. Sychov R.O. - Associate Professor of the Department of Internal Diseases №3, Zaporozhye State Medical University, MD, PhD
3. Tokarenko I.I. - Assistant of the Department of Internal Diseases №3, Zaporozhye State Medical University, MD, PhD
4. Shevchenko M.V. - Assistant of the Department of Internal Diseases №3, Zaporozhye State Medical University, MD, PhD
5. Chornaya I.V. - Associate Professor of the Department of Internal Diseases №3, Zaporozhye State Medical University, MD, PhD
6. Kulynych O.V. - Associate Professor of the Department of Internal Diseases №3, Zaporozhye State Medical University, MD, PhD

Reviewers:
V. G. Deynega Honored Worker of Science and Technology of Ukraine, Doctor of Medical Sciences, Professor of Department of Family Medicine and Therapy of FPE
O. V. Kraydashenko Doctor of Medical Sciences, Professor, Head of Department of Clinical Pharmacology, Pharmacy and Pharmacotherapy with the Course of Cosmetology

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Навчальний посібник **КЛІНІЧНА ІМУНОЛОГІЯ ТА АЛЕРГОЛОГІЯ** розроблений для підготовки до практичних занять англомовним студентами, які навчаються на 5-му курсі.

CONTENTS

FOREWORD

Clinical immunology is a discipline with a distinguished history, rooted in the prevention and treatment of infectious diseases in the late nineteenth and early twentieth centuries. The conquest of historical scourges such as smallpox and (substantially) polio and relegation of several other diseases to the category of medical curiosities is often regarded as the most important achievement of medical science of the past fifty years. Nevertheless, the challenges facing immunologists in the efforts to control infectious diseases remain formidable; HIV infection, malaria and tuberculosis are but three examples of diseases of global import that elude control despite major commitments of monetary and intellectual resources. Although firmly grounded in the study and application of defenses to microbial infection, since the 1960s clinical immunology has emerged as a far broader discipline. Dysfunction of the immune system has been increasingly recognized as a pathogenic mechanism that can lead to an array of specific diseases and failure of virtually every organ system. Pardoxically, although the importance of the immune system in disease pathogenesis is generally appreciated, the place of clinical immunology as a practice discipline has been less clear. As most of the non-infectious diseases if the human immune system lead eventually to failure of other organs, it has been organ-specific subspecialists who have usually dealt with their consequences. Recently, however, the outlook has begun to change as new diagnostic tools increasingly allow the theoretical possibility of intervention much earlier in disease processes, often before irreversible target organ destruction occurs. More importantly, this theoretical possibility is increasingly realized as clinical immunologists find themselves in the vanguard of translating molecular medicine from laboratory bench to patient bedside. In many settings, clinical immunologists today function as primary care physicians in the management of patients with inmune-deficiency, allergic, and autoimmune diseases. Indeed many influential voices in the clinical disciplines of allergy and rheumatology support increasingcoalescence of these traditional subspecialities around their intellectual core of immunology. In addition to his or her role as a primary care physician, the clinical immunologist is increasingly being looked to as a consultant, as scientific and clinical advances enhance his or her expertise. The immunologist with a ‘generalist’ perspective can be particularly helpful in the application of unifying principles of diagnosis and treatment across the broad spectrum of immunologic diseases.

THEME №1. STRUCTURE AND PRINCIPLES OF FUNCTION OF THE IMMUNE SYSTEM. THE AGING IMMUNOLOGY

It took more than 400 million years of evolution for our immune system to develop into the highly complex and adaptable defense mechanism that it is today. Its primary task is to protect us from foreign and harmful substances, microorganisms, toxins, and malignant cells. Only through the continuous development ofthe immune system was it possible to protect living organisms against constant attacks from both the external and internal environments. In the process, the immune system has learned to inactivate destructive responses to endogenous substances and to prevent irreparable damage to the surrounding tissue. Most immunological responses are of limited duration and are restricted by regulatory mechanisms to prevent overreactions.

An essential task of the immune system is to distinguish dangerous from harmless. Infiltration with microorganisms or bacterial toxins, for example, is a dangerous attack on an organism, whereas the inhalation of pollen or the infiltration offood antigens from the stomach into the blood system is harmless. The destruction of malignant cells or foreign cell material is desirable (e.g., in parasite infestation), but direct at-tacks against the host tissue are undesirable (e.g., in autoimmune disease). The processes by which the immune system avoids the development of destructive self-reactivity are collectively referred to as tolerance. The large majority of lymphocytes directed against self-antigens present throughout the primary lymphoid organs are destroyed in a process known as central tolerance. Peripheral tolerance is still another mechanism that occurs in less common endogenous structures or in those present only in certain regions of the body.

Nonspecific (innate) immune system. The historically older congenital defense mechanisms are defined as nonspecific because they become active independently of the invading pathogen. They are also called nonclonal defense mechanisms because no individual cell clone is required for their specific development. ***Some examples include*** the acid layer of the skin, the intact epidermis, the complement system, antimicrobial enzyme systems, and non-specific mediators such as interferons and interleukins. ***Examples on the cellular level*** include granulocytes, the monocyte-macrophage system, and natural killer (NK) cells. The latter represent an interface between the specific and nonspecific immune systems.

The inflammatory response permits an on-the-site concentration of defensive forces via the complex interplay of soluble and cellulareomponents; this is an important nonspecific defense mechanism. The first step in this process is the release of mediators that dilate the blood vessels and make the capillary walls more permeable. The site of infection is then pe-netrated by granulocytes, which are replaced by macrophages in the later course of the reaction The granulocytes carry out the "first line of defense" in which the majority of invading pathogens are destroyed. The remaining pathogenic organisms and waste products of this firstline defense are phagocytosed by macrophages.

Specific (adaptive) immune system. The process ofsuch an immune response paves the way for the specific immune response. In a specific cytokine environment, the body can decide whether to proceed to a more humoral line of defense or a more cellular line of defense. The migration of antigen-presenting cells (APC) to the lymphoid organs first triggers a systemic immune response, then a memory response. The ***specific immune system consisting*** of T and B lymphocytes is responsible for this. These cell systems can produce highly specific reactions to their respective antigens and undergo clonal expansion, thus achieving a highly effective response to and memory for those antigens.

Origin of Cells of the Immune System Cells of the immune system originate in the bone marrow. Once mature these cells patrol tissues, circulating in blood and lymphatic vessels. Haematopoietic stem cells (HSCs) found mainly in the bone marrow give rise to all blood cells and cells of the immune system. HSCs differentiate into both myeloid and lymphoid progenitors (Figure 1. 1).

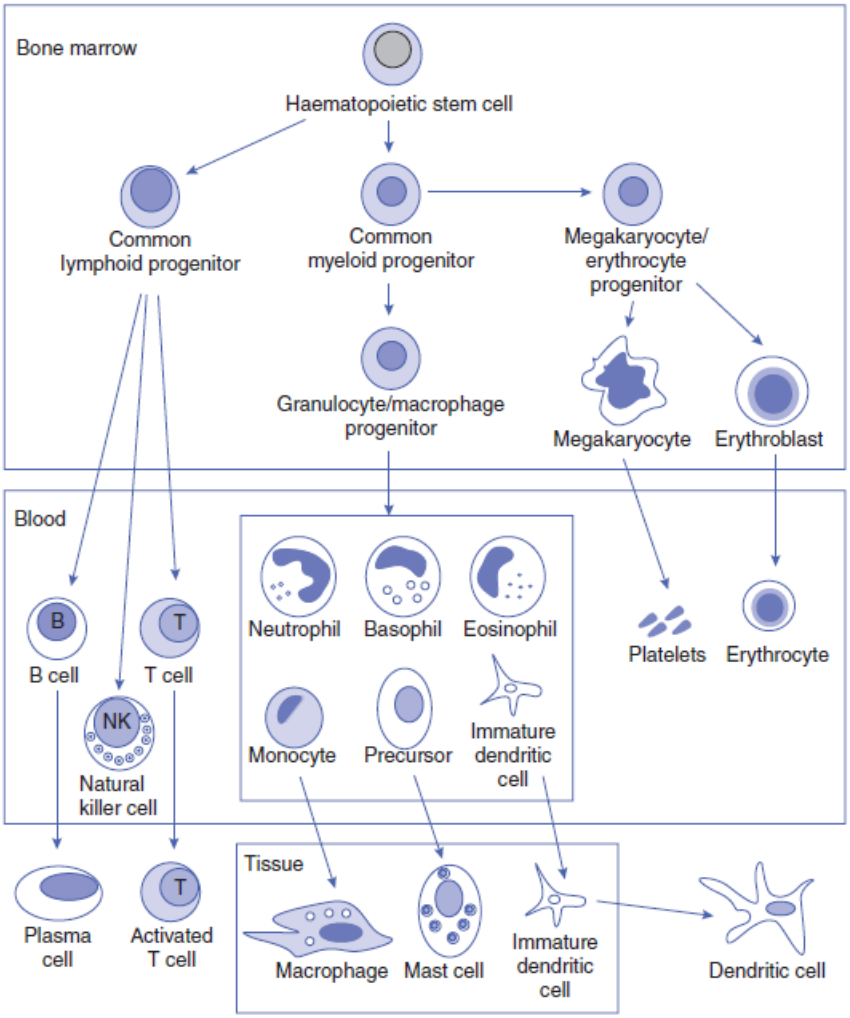

Figure 1. 1 Origin of blood cells

MYELOID CELLS

Myeloid cells are involved in innate and adaptive immunity. They include granulocytes (neutrophils, eosinophils, basophils), monocytes and macrophages, dendritic cells and mast cells.

Granulocytes. Granulocytes have densely staining cytoplasmic granules and have multilobcd nuclei (hence also known as polymorphonuclear leucocytes or PMNs).

Neutrophils are short-lived, mobile phagocytes, which circulate in the bloodstream until recruited to sites of inflammation. They are the most numerous cells of the innate immune response, but also interact with antibodies playing an effector role in adaptive responses. Eosinophils play a role in defence against parasitic infections and are also recruited to sites of allergic inflammation. The function of basophils is uncertain.

Monocytes and macrophages. Monocytes circulate in the blood, migrate into tissues and differentiate into macrophages. Macrophages are part of the mononuclear phagocyte system (previously known as the reticuloendothelial system) and are distributed widely in body tissues. Macrophages play an important role in innate immunity and also present antigen to T cells.

Dendritic cells. Dendritic cells are professional APCs, which migrate from blood into tissues. On encountering a pathogen, dendritic cells ingest antigen by phagocytosis, mature and migrate to lymph nodes where they present antigen to T cells and activate T cells.

Mast cells. Mast cells are found in connective tissue and close to mucosal surfaces. They trigger local inflammatory responses to antigen by rapidly releasing inflammatory mediators including histamine. Mast cells play a pivotal role in allergic responses.

LYMPHOID CELLS

The common lymphoid precursor matures into lymphocytes, including B and T cells which generate adaptive immune responses.

B cells and T cells. B cells develop in the bone marrow. T cell precursors leave the bone marrow and mature in the thymus. B and T lymphocytes cannot be distinguished morphologically - both are small cells with condensed chromatin and few cytoplasmic organelles. B and T lymphocytes can be distinguished by assessing expression of lineage-specific molecules. Mature B and T cells circulate between the blood and peripheral lymphoid tissues. Encounter with antigen triggers proliferation and differentiation into cells with specialised effector functions. Both B and T cells have a large repertoire of receptors that can recognise a wide diversity of antigens.

Plasma cells. B cells mature into plasma cells, terminally differentiated cells which secrete antibody.

NK cells. NK cells are large granular lymphocytes found throughout the tissues of the body but predominantly in the circulation. They lack antigen-specific receptors and are part of the innate immune system. NK cells kill tumour cells and virus-infected cells.

LYMPHOID ORGANS

Lymphoid organs are specialised tissues where lymphocytes develop, mature and differentiate. They are divided into primary lymphoid organs, where lymphocytes develop, and secondary lymphoid organs, where adaptive immune responses are initiated (Figure 1.2).

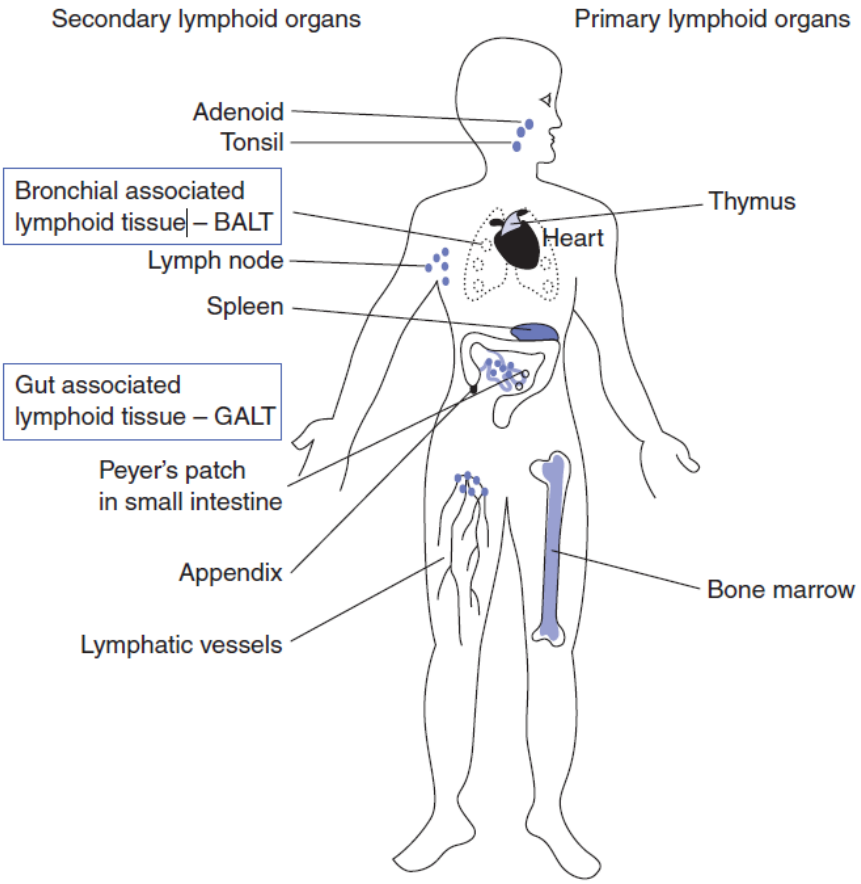

Figure 1. 2 Primary and secondary lymphoid organs.

Primary lymphoid organs - bone marrow and thymus. B and T cells originate in the bone marrow but only B cells mature here (bone marrow derived). T cells migrate to the thymus for maturation (thymus derived). Once maturation is complete, both types of cells enter the bloodstream and migrate to the peripheral lymphoid organs.

Secondary lymphoid organs. Secondary lymphoid organs are organised to trap antigen from sites of infection, facilitate antigen presentation to lymphocytes and provide the optimal microenvironment for lymphocyte maturation. They include lymph nodes, spleen and mucosa-associated lymphoid tissue (MAI.T) and all share the same basic structure. Immune responses are initiated in secondary lymphoid tissues.

Lymph nodes. Lymph nodes trap antigens from sites of infection in tissue (Figure 1. 3). Afferent lymphatic vessels transport extracellular tissue fluid (lymph) carrying antigen and APCs to the lymph nodes. In lymph nodes, B cells are concentrated in follicles and T cells are distributed in the surrounding paracortical area (T cell zone). When B cells encounter their specific antigen and antigen-specific helper T cells, they proliferate in germinal centres of the follicles. The organisation of lymph nodes and other secondary lymphoid tissues promotes B cell interaction with helper T cells, which is essential for antibody responses.

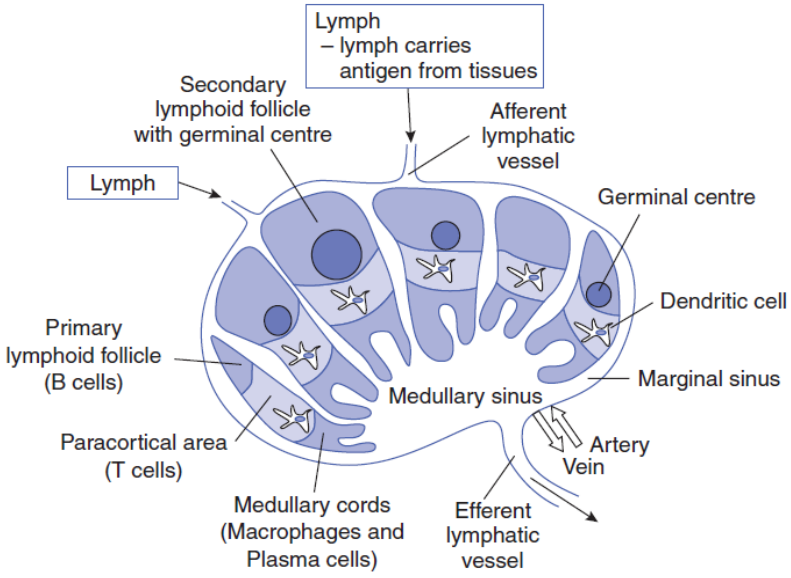

Figure 1. 3 A lymph node.

Spleen. The spleen collects antigens from the blood. The main bulk of the spleen is red pulp, the site of red blood cell (RBC) disposal. Lymphocytes surround arterioles entering the spleen forming areas of white pulp, which are divided into the peri-arteriolar lymphoid sheath (PALS), containing mostly T cells, and the B cell corona. Lymphocytes and antigen-loaded dendritic cells come together in the PALS.

Mucosal-associated lymphoid tissue. MALT includes the gut-associated lymphoid tissue (GALT), bronchial-associated lymphoid tissue (BALT) and aggregates of lymphocytes in other mucosae. GALT collects antigen from the epithelial surfaces of the gastrointestinal tract (GIT) and includes the tonsils, adenoids, appendix and specialised structures called Peyers patches in the small intestine.

The mucosal surfaces are particularly vulnerable to infection. The immune system must avoid responding to food antigens, while still detecting and killing pathogens. The MALT lining the gut is known as gut-associated lymphoid tissue or GALT and includes tonsils, adenoids and Peyer’s patches.

PEYER'S PATCHES Peyer's patches facilitate induction of immune responses in the small intestine. Specialised epithelial cells called M cells form a membrane overlying the lymphoid tissue and take up antigens from the gut lumen by endocytosis. Antigens are transported through M cells and delivered directly to APCs (dendritic cells) and lymphocytes of the mucosal immune system.

A DISTINCTIVE REPERTOIRE OF LYMPHOCYTES There are small foci of lymphocytes and plasma cells scattered widely throughout the lamina propria of the gut wall. These are effector cells of the gut mucosal immune system and the T cells can be divided into conventional αβ-T cells and γδ-T cells.

Naive lymphocytes leave the thymus and enter the mucosal immune system via the bloodstream. On encountering foreign antigens, lymphocytes are activated and traffic, via the lymphatics through mesenteric lymph nodes, to the thoracic duct and circulate in blood throughout the entire body. They re-enter mucosal tissues including other sites of MALT (respiratory and reproductive mucosa). Hence immune responses initiated in Peyer's patches are disseminated throughout mucosal sites. This pathway of lymphocyte trafficking is distinct from and parallel to that of lymphocytes in the rest of the lymphoid system.

SECRETORY IgA The major antibody isotype present in the lumen of the gut is secretory polymeric IgA, synthesised by lamina propria plasma cells and transported into the gut lumen. Polymeric IgA binds mucus overlying the gut epithelium, acting as an antigen-specific barrier to pathogens and toxins.

MOST ANTIGENS PRESENTED TO THE MUCOSAL IMMUNE SYSTEM INDUCE TOLERANCE The mucosal lymphoid system is exposed to many foreign antigens from foods and commensal bacteria to pathogenic microbes and parasites. Immune responses to food antigens are rarely detected. Feeding foreign antigens leads to specific, active unresponsiveness - known as oral tolerance. In contrast, pathogenic microorganisms induce strong TH1 responses.

The context in which peptide is presented to T cells of the mucosal immune system appears to determine whether tolerance or a powerful adaptive immune response ensues. In the absence of inflammation, presentation of peptides to T cells by MHC molecules on APCs occurs without adequate co-stimulation. However, pathogenic organisms induce inflammatory responses, which stimulate maturation and expression of co-stimulatory molecules on APCs. Subsequent antigen presentation favours development of a TH1 response.

Lymphocyte trafficking Lymphocytes express adhesion molecules, with which they attach to endothelial cells prior to migrating into tissues or lymphoid organs. Some lymphocytes have specific adhesion molecules (addressins), which bind to ligands on endothelial cells in particular vascular beds, for example, mucosal lymphocytes express addressins which favour migration into MALT.

Naïve T and B cells are mature lymphocytes that have not yet encountered antigen. These cells continually migrate into the secondary lymphoid tissues via the bloodstream. They return to the blood via the (efferent) lymphatic vessels and the thoracic duct. Naïve lymphocytes recirculate until they meet their cognate antigen. If this docs not occur they die.

When pathogens enter the body, APCs (c.g. dendritic cells) take up and process antigen. APCs and free antigen are carried in afferent lymphatics to regional lymph nodes. Here APCs display processed antigen to recirculating T lymphocytes, activating antigen-specific cells. Activated T cells proliferate and differentiate into antigen-specific effector cells and leave the lymph node via efferent lymphatics, re-enter the blood stream and migrate to the site of infection.

Recirculating B cells that encounter antigen are activated, proliferate and differentiate into antibody-secreting plasma cells. Plasma cells may remain in the lymph node or return to the bone marrow (via efferent lymphatics and bloodstream).

All secondary lymphoid tissues trap APCs and antigen, present it to migratory lymphocytes thus stimulating an adaptive immune response. B cell follicles of the lymph nodes expand and proliferate to form germinal centres and the entire lymph node enlarges - giving rise to swollen glands.

LYMPHOCYTE POPULATIONS

B lymphocytes B cells recognise antigens through surface immunoglobulin (sIg). Terminally differentiated B cells, called plasma cells, produce and secrete antibodies, the specificity of which is identical to the sIg expressed by their B cell precursors. Following maturation in the bone marrow, most B cells that survive the selection process circulate through secondary lymphoid tissues. These are conventional or B-2 B cells.

B-1 B cells are a smaller subset of B cells, which produce a limited repertoire of low affinity and polyspecific antibodies reactive with common bacterial antigens. B-1 B cells may represent a primitive population whose function is intermediate between innate and specific immune responses.

T lymphocytes T lymphocytes express T cell receptors (TCRs) and CD3. Most T lymphocytes express a TCR comprising a and β chains (αβ T cells) and also either CD4 or CD8, which direct

LYMPHOCYTE MATURATION the pattern of antigen interaction. Antigens recognised by αβ T cells are processed peptides, presented by host-derived MHC molecules. Factors influencing the pattern of T cell response include the pattern of innate immune activation, characteristics of the stimulating antigen, and type of antigen presentation.

αβ T cells

Two broad categories of αβ T cells are described as follows:

♦Cytotoxic T cells (CTLs or Tcs) - usually express CD8 and recognise endogenous antigens (viruses, tumours).

♦Helper T cells (THs) usually express CD4. They respond to extracellular antigens including bacteria that are taken up into vesicles in the cells. Activation of helper T cells requires processing and presentation of antigen in association with MHC Class II molecules by professional APCs. These cells typically 'help' other cells of the immune system rather than having a direct effector function.

Helper T cells are in turn further sub-divided based on patterns of cytokine expression. The two extremes are:

♦ TH1 - produce IFN-y and tumour necrosis factor that enhance macrophage function, cellular immunity, production of some antibody types and granulomatous inflammation.

♦ TH2 - enhance IgE-mediated responses through the action of IL-4 and IL-5.

TH1 and TH2 responses are for the most part mutually exclusive and suppressive of the other. Further T cell subpopulations are increasingly recognised, such as regulatory T cells - TR - and TH3 that impose control on cell-mediated immune responses through the action of cytokines including IL-10 and TGF-β.

γδ T cells γδ T cells express a TCR composed of y- and δ-chains and are preferentially expressed in skin and at mucosal surfaces. Their antigen interactions are not as specific as those of αβ T cells. Diverse effector functions mirroring those of αβ T cells have been identified. These cells may represent a T cell equivalent of B-l B cells.

Unconventional T cells CD3+ T cells expressing unusual patterns of surface molecules common to conventional T cells and NK cells are called NK-T cells. Antigen recognition by NK-T cells extends beyond peptide fragments expressed by MHC molecules. The functional significance of this population is not fully understood.

NK cells These can kill certain tumour targets and virus-infected cells. Early production of IL-12 by NK cells encountering viruses and intracellular bacteria skews the later specific immune responses towards a THl-type response.

NK cells express activating receptors and inhibitory receptors, which interact with MHC Class I molecules, preventing uncontrolled activation of NK cells. If MHC expression is disturbed, as occurs in virus-infected or tumour-laden cells, inhibition is lost resulting in NK cell cytotoxicity.

THE DEVELOPMENT OF MATURE LYMPHOCYTE POPULATIONS Haemopoietic stem cells (HSCs) differentiate into myeloid and lymphoid progenitor cells. Lymphoid-committed precursor cells mature in

♦Bone marrow - where they receive signals that promote development into B cells or

♦Thymus - where T cells develop.

A small proportion of T cells, especially non-conventional ones like γδ T cells and NK-T cells may mature outside the thymus.

Lymphocytes mature by a series of sequential differentiation steps defined by patterns of molecular expression, especially antigen receptor or antigen receptor-associated molecules. The early phases of lymphocyte maturation occur independent of exposure to antigen. Once complete antigen receptors are expressed by developing lymphocytes, further survival and functional capacity requires interaction with antigen (Figure 1.4).

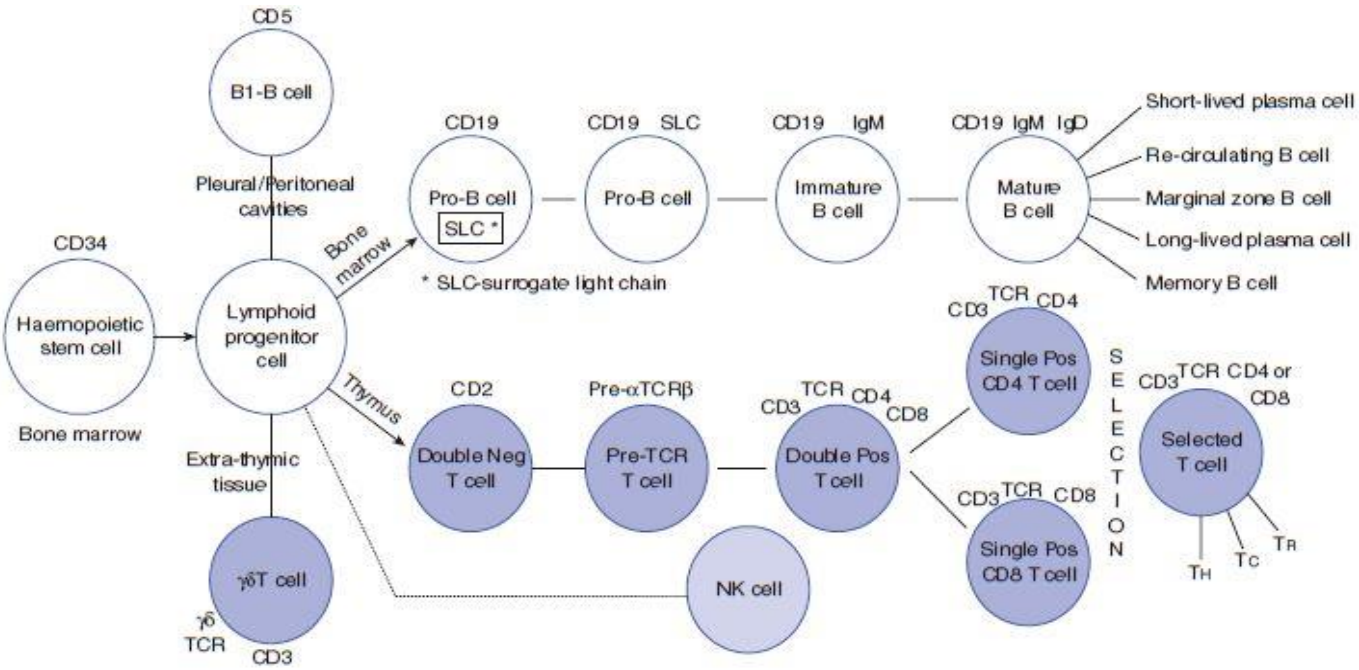

Figure 1. 4 Lymphocyte maturation.

B lymphocyte maturation B cells are generated throughout life in the bone marrow. Development stages are marked by the ordered rearrangement of immunoglobulin genes. The key stages and the important molecule expression patterns are highlighted.

♦***Pro-B cells*** - express B cell-specific molecules (CD 19, surrogate light chain). Immunoglobulin genes are in germline configuration.

♦***Pre-B cells*** - express surface IgM heavy chain associated with surrogate light chain - pre-B receptor. Immunoglobulin genes are rearranged by enzymes encoded by Recombinase Activating Genes (RAG), and the IgM expressed originates from whichever chromosome successfully rearranged first. RAG gene expression is switched off, inhibiting rearrangement of immunoglobulin heavy chain genes on the other chromosome - allelic exclusion. Subsequent re-expression of RAG allows light chain immunoglobulin genes to rearrange. Kappa (K) genes initially rearrange. If unsuccessful, the λ genes rearrange sequentially until a functional light chain is produced.

♦***Immature B cells*** - IgM+ IgD— immunoglobulin receptor expression allows these cells interact specifically with antigen. Further survival and differentiation is dependent on whether or not selection or activation signals are received through the BCR.

T lymphocyte maturation T cells develop from thymic lymphoid progenitor cells. NK cells and NK-T cells also arise from thymic progenitors, but details of their development are unclear. As with B cells, stages of T cell maturation are marked by specific patterns of TCR and other molecule expression. The key stages are

♦***'Double-negative' cells*** - lack CD4 and CD8 expression. Most have not rearranged TCR gene segments. CD2 but not CD3 is expressed.

♦***'Double positive' cells*** - dually express CD4 and CD8. T cells only reach this stage if a successful TCR is made.

♦ ***Selected T cells*** - at this stage T cells are screened for ability to interact with host MHC - peptide complexes expressed on cortical epithelial cells and interdigitating dendritic cells. They also lose either CD4 or CD8 and become 'single positive' thymocytes. T cells must be able to recognise MHC for conventional T cell-mediated antigen recognition. Cells that pass this hurdle are positively selected for survival. T cells then undergo negative selection, where potentially autoreactive T cells are eliminated, an important safeguard against autoimmunity.

Negative selection in T cells is more rigorous than with B cells. A lack of T cell help will hold autoreactive B cells in check. Only about 1% of thymocytes mature and enter the peripheral pool.

Both αβ- and γδ-T cells are derived from the same early thymocyte precursor cell type. The type of TCR expressed - either TCR^ or TCRyS - depends on which of the TCR chains makes the first successful rearrangement. The same T cell cannot express both types of TCR. Successful rearrangement of TCRa disables δ-gene segments, and β and δ chains do not usually align correctly to form a functional TCR. Early in life, more T cells of the TCRγδ lineage are produced, but subsequently TCRαβ-expressing T cells predominate. TCRγδ cells often do not express either CD4 or CD8.

IMMUNOGLOBULIN STRUCTURE

Antibodies, also called immunoglobulins (Ig), are glycoproteins, produced by plasma cells, that bind antigens with a relatively high specificity and affinity.

ANTIBODY STRUCTURE Antibody molecules are comprised of two identical light chains and two identical heavy chains. Light chains may be kappa (K) or lambda (λ) in type, but this does not affect antibody function.

The antibody isotype or class is determined by the heavy chain (μ, γ, α, ε or δ) giving rise to immunoglobulin M (IgM), immunoglobulin G (IgG), immunoglobulin A (IgA), immunoglobulin E (IgE) and immunoglobulin D (IgD), respectively. In addition to differences in the basic antibody unit, antibody isotypes differ in the number of units in a typical molecule - IgM is usually pentameric, IgA dimeric and both IgG and IgE monomeric (Figure 1.5). The five isotypes differ greatly in functional activity. IgG is subdivided into the subclasses IgG1, IgG2, IgG3 and IgG4 and IgA into IgA1 and IgA2 subclasses. Antibody subclasses differ in heavy chain amino acid sequences conferring functional differences.

Variable and constant regions of heavy and light chains Heavy and light chains have a variable region (composed of variable domains), and a constant region (composed of constant domains). Variable regions of heavy and light chains generate two identical antigen-binding sites, conferring specificity on the antibody. The heavy-chain constant region determines the antibody isotype and functional properties.

Functionally distinct fragments of the antibody molecule - Fc and Fab

Proteolytic cleavage by the enzyme papain cleaves immunoglobulin into two Fab fragments and one intact Fc fragment. The Fab fragment binds antigen and the Fc fragment contains constant domains.

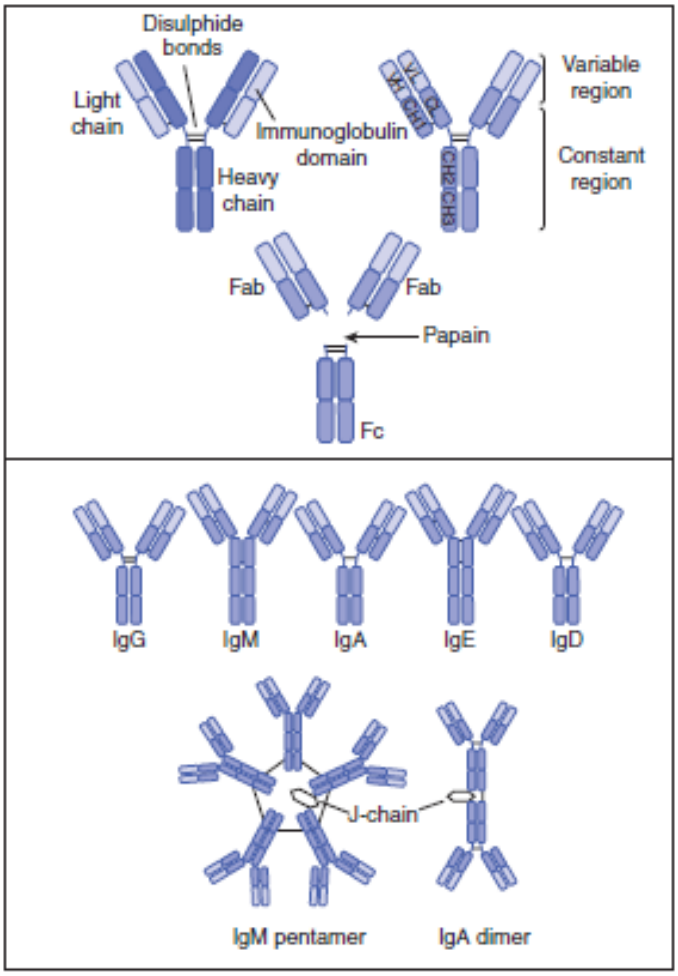

Figure 1. 5 Structure of immunoglobulin molecules.

ALLOTYPES Non-functional polymorphisms occur in genes encoding heavy chain constant regions giving rise to allotypes. Allotypes are differences between individuals' antibody molecules, comparable to different blood groups. There is no functional difference between antibodies of different allotypes.

IDIOTYPES Idiotypes are unique antigenic determinants found in antigen-binding sites of antibodies. They result from different amino acid sequences in antigen-binding sites that determine the antibody specificity. Idiotypes are unique to antibodies produced by the same clone of B cells.

GENERATION OF DIVERSITY The antibody response to an antigen is diverse, generating many different B cell clones each with its own unique specificity. An individual's collection of antibody specificities is called

the antibody repertoire. Antibody diversity is generated during B cell development by random combination of gene segments from heavy-chain and light-chain gene groups (Figure 1.6).

There are three gene clusters encoding immunoglobulins:

♦κ-chain clusters are found on chromosome 2

♦λ-chain genes are found on chromosome 22

♦Heavy-chain gene clusters are found on chromosome 14.

Variable and constant regions of immunoglobulin molecules are encoded by V gene segments and C gene segments, respectively.

Somatic recombination generates variable regions The variable region of heavy and light chains is generated from separate V region gene segments by a process of DNA rearrangement known as somatic recombination. The heavy-chain V region gene segments include Variable (VH), Diversity (DH) and Joining (JH), while the light chain contains variable (VL) and joining segments (JL).

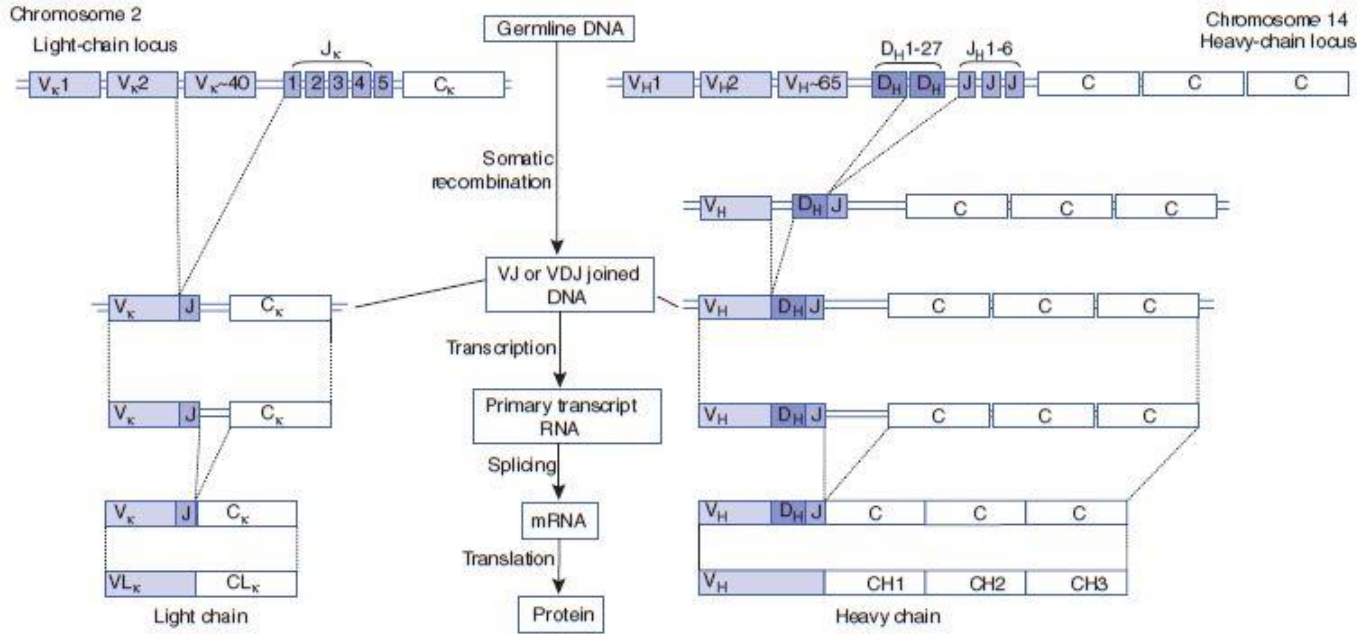

Figure 1. 6 The production of immunoglobulin molecule heavy and light chains: somatic recombination, RNA splicing and production of protein. Rearrangement of κ light chain is used to illustrate the process, however, λ chain rearrangement follows identical steps.

V region recombination involves the 'VDJ recombinase' enzyme complex, which includes generic DNA cleavage and repair enzymes and lymphocyte-specific components. RAG-1 and RAG-2 are lymphocyte-specific enzymes, essential for VDJ recombination. VDJ recombination results in removal of intervening gene segments, permanently changing the genomic DNA in the B cell. The assembled variable regions of both heavy and light chains join to their respective constant regions after transcription, by RNA splicing. Heavy- chain C region genes have several gene segments, each encoding the constant region for a different antibody class (Cg, Cy, Ca, C8, Ce). κ chains have one C gene segment for its constant region, while λ chains have four C gene segments. Heavy chain genes undergo rearrangement before light chain genes.

Regulation of immunoglobulin gene rearrangement ensures that each B cell only expresses one rearranged heavy chain and one rearranged light chain. Therefore, each B cell expresses millions of identical surface antibodies.

ANTIBODY DIVERSITY IS GENERATED BY FOUR PROCESSES, WHICH TOGETHER CREATE A VAST REPERTOIRE OF ANTIBODY SPECIFICITIES FROM A LIMITED NUMBER OF GENES

1 There are several different gene segments making up the variable regions of both heavy and light chains (Table 1. 1). Different combinations of variable region gene segments recombining randomly generate considerable diversity in the antigen-binding site. This is known as combinatorial diversity.

2 Pairing of different heavy and light chains increases diversity.

3 During recombination, imprecise joining of gene segments results in insertion of additional nucleotides - known as junctional diversity.

4 Somatic hypermutation introduces point mutations into variable regions of rearranged heavy and light chain genes. This allows the antibody specificity to be changed after recombination occurs. Mutant antibody molecules may bind antigen better or less well than the original antibody. B cells expressing higher affinity antibody are selected and mature into antibody-secreting cells. Somatic hypermutation and selection of high affinity antibody gives rise to the physiological phenomenon of affinity maturation, whereby the affinity of antibodies produced improves as an immune response develops.

Table 1. 1 Gene segments making up the variable regions of heavy and light chains

GENE SEGMENT	LIGHT CHAIN (κ)	LIGHT CHAIN (λ)	HEAVY CHAIN (H)
Variable (V)	40	30	65
Diversity (D)	0	0	27
Joining (J)	5	4	6

Rearrangement of gene segments occurs in developing B cells in bone marrow. Somatic hypermutation occurs in B cells in secondary lymphoid organs after functional antibody has been expressed.

THE B CELL RECEPTOR COMPLEX Immunoglobulin was first discovered in plasma. However, before antibody can be secreted, immunoglobulin must function as the cell-surface antigen receptor, that is, the B cell receptor (BCR). Antigen binding to BCR activates B cells, leading to clonal expansion and differentiation into antibody-secreting plasma cells. B cells initially express transmembrane IgM and when activated differentiate into plasma cells. B cell activation usually requires specialised interactions with helper T cells that provide additional signals.

The BCR complex includes the antigen receptor, surface immunoglobulin, associated with two other polypeptides, Iga and Igp. When sIg is crosslinked, these proteins transmit signals leading to B cell proliferation and differentiation. They are also required for the expression and assembly of immunoglobulin (Figure 1.7). B cell co-receptors (CD21, CD19 and CD81) play an important role in enhancing or inhibiting B cell activation.

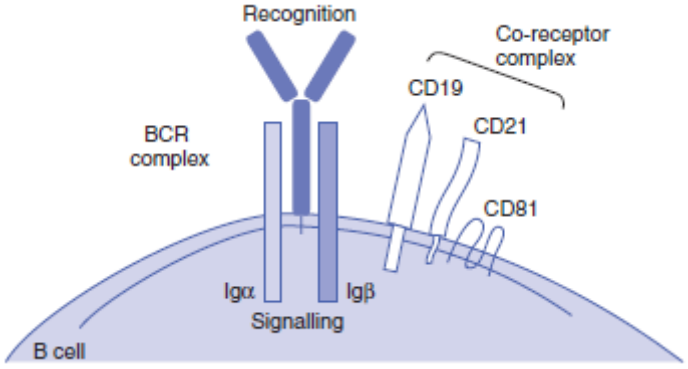

Figure 1.7 The B cell receptor complex and its co-receptor complex.

IMMUNOGLOBULIN FUNCTION

Antibody binding to pathogen is not sufficient to kill the organism. The Fc portion of immunoglobulin (Ig) can recruit complement or phagocytes to kill the organism. In this way antibodies harness innate effector mechanisms to kill microbes. Antibody protects by

- ♦Neutralising toxins
- ♦Neutralising organisms
- ♦Activating complement
- ♦Opsonising organisms.

NEUTRALISING TOXINS Toxins bind to receptors on cells, altering cell function. If antibody prevents interaction of toxin with receptor, cell function remains unaffected and the toxin is neutralised. Neutralisation is dependent on the antigen-binding domain only, and is not affected by the Fc portion of Ig (Figure 1.8).

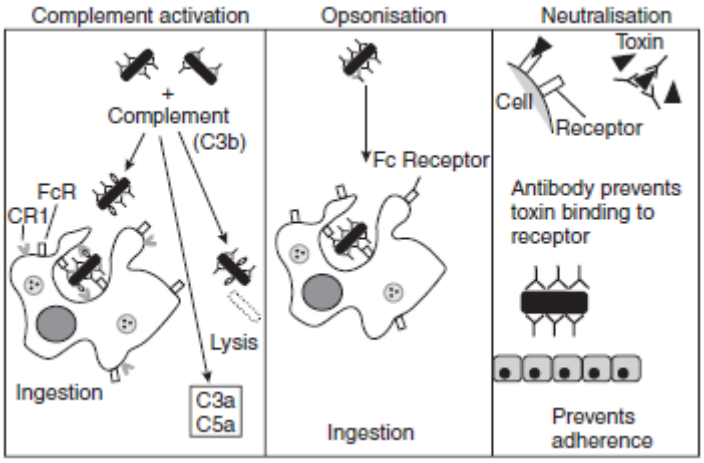

Figure 1.8 Antibody functions.

NEUTRALISING ORGANISMS To establish infection organisms must adhere to epithelial surfaces using a limited number of adhesion molecules. Antibodies that bind to key portions of these adhesion molecules prevent organisms binding to mucosae and subsequent infection. Neutralisation of organisms is only affected by the antigen-binding domain, and not by the Fc portion. However, the antibody isotype determines the ability of antibody to cross mucosal surfaces.

COMPLEMENT ACTIVATION IgG and IgM activate complement via the classical pathway. This generates anaphylatoxins (C3a and C5a) that activate mast cells and attract neutrophils to the site of infection.

Deposition of C3 on the organism results in assembly of the membrane attack complex (MAC). In susceptible organisms, insertion of MAC causes leakage of cellular contents and lysis. Bacterial capsules may inhibit MAC assembly, conferring resistance to complement- mediated lysis. Complement activation requires the Fc portion of Ig, and the effectiveness of antibodies at activating complement varies with the isotype.

OPSONISATION Opsonisation is the process whereby microbes and other cells are flagged for phagocytosis. Phagocytes (neutrophils and macrophages) ingest some organisms in the absence of antibody, however, this is relatively inefficient. Antibody-coated organisms are usually phago- cytosed. The antibody acts as a bridge between the organism and Fc receptor on the phagocyte. Binding of Ig to the Fc receptor stimulates phagocytosis and the respiratory burst. Fc receptor activation renders the phagocyte competent to kill most pathogens. Opsonisation requires the Fc portion of the antibody molecule.

Complement (particularly C3b) deposition acts synergistically with antibody to greatly improve opsonisation. C3 fragments bind complement receptors on phagocytes, stimulating particle uptake and activation of neutrophils and macrophages. Co-stimulation of neutrophils through Fc and C3 receptors results in greatly enhanced activation.

ANTIBODY AFFINITY Antibody affinity is the binding strength of an antibody-binding site to an antigen. High affinity results in tight binding and less chance of antibody dissociating from antigen. Different antibody molecules produced in response to the same antigen vary in their tightness of binding. Antibodies produced by a memory response have higher affinity than in a primary response.

ANTIBODY AVIDITY An antibody's avidity is determined by the combined binding strength of all antigen-binding sites. More binding sites result in increased total binding strength or avidity of antibody binding to antigen.

IMMUNOGLOBULIN ISOTYPE AND FUNCTION

Antibody isotypes have functionally distinct properties, determined by the immunoglobulin constant regions. The constant region recruits cells and molecules to help destroy pathogens. Additionally, each

antibody class has different biological activities, capable of dealing with different microbes at different sites (Table 1.2).

Table 1.2 Immunoglobulin isotype function and distribution

IMMUNOGLOBULIN	**IgG**	**IgA**	**IgM**	**IgD**	**IgE**
Molecular weight (kDa)	146	160	970	184	188
Functional activity					
Complement activation (classical path)	+ + +	-	+++	-	-
Complement activation (alternative path)	-	+	-	-	-
Neutralisation	+ +	+ +	+	-	-
Opsonisation	+ + +	+	-	-	-
Sensitisation for killing by NK cells	+ +	-	-	-	-
Sensitisation of mast cells	+	-	-	-	+++
Distribution					
Transports across epithelium	-	+ + + dimer	+	-	-
Transports across placenta	+ + +	+/-	-	-	-
Diffusion into extravascular spaces	+ + +	+ + monomer	+ /-	-	+
Mean serum level (mg/ml) adults	13-15	2.1	1.5	0.04	$3x10^{-5}$
Half-life in serum (days)	21	6	10	3	2

IgG IgG is the most abundant antibody and is found in extracellular fluid. IgG crosses the placenta providing passive immunity that persists for 3-6 months after birth. IgG1, IgG2, IgG3 and IgG4 have slightly different sequences in their heavy chains and corresponding differences in their functions.

IgA IgA is the main immunoglobulin in secretions (colostrum, saliva etc.) where it exists as a dimer. IgA is secreted locally by plasma cells in the mammary and salivary glands, and along the respiratory, gastrointestinal and genitourinary tracts. IgA is transported through the epithelial cells into the lumen. Secretory IgA contains a secretory component (SC) and a J-chain (joining chain) required for transepithelial transport and stability of the IgA molecule. IgA is the major component of the adaptive immune response at mucosal surfaces.

IgM IgM is the first antibody produced by B cells. It is expressed on B cells as the B cell antigen receptor. Pentameric secreted IgM is also found in blood. IgM antibodies usually have low affinity antigen-binding sites, however, since IgM has 10 antigen binding sites per molecule, the overall binding avidity is high. This pentameric structure makes it very effective for activating the complement system.

IgD IgD is present in low quantities in the circulation. The principal known function is as an antigen receptor on B cells. B cells can express both IgM and IgD, which have the same antigen specificity.

IgE IgE is present in the serum in nanogram quantities. IgE plays an important role in protection from infection by parasites and in immediate hypersensitivity responses.

CYTOTOXIC T CELLS

Cell mediated immunity involves two separate populations of T cells: ***helper T cells (THs) and cytotoxic T cells (TCs)***. Viruses and some bacteria replicate in the cytoplasm of infected cells where they are inaccessible to antibodies. Cytotoxic T cells kill these infected cells either by inducing them to undergo programmed cell death (apoptosis) or by necrosis. Cytotoxic T cells also play a role in graft rejection and tumour immunity.

Apoptosis results in DNA fragmentation and destruction of the cell from within. Apoptotic cells are rapidly ingested and digested by phagocytes, allowing efficient removal of cells without an inflammatory

response. Cell death by necrosis results in inflammation. These mechanisms prevent the release of infectious viruses to infect other healthy cells.

THEME №2. IMMUNOLOGICAL RESEACH METHODS. THE CONCEPT OF IMMUNOGRAM

INITIAL APPROACH TO THE PATIENT

Immune deficiency disorders should be considered once the more common causes of recurrent infection have been excluded. The initial approach to a child or adult with recurrent infections is described separately.

Immune dysregulation can result in disorders other than recurrent infections, including:

- Autoimmune disorders, such as autoimmune hemolytic anemia
- Inflammatory disorders, such as inflammatory bowel disease or inflammatory arthritis
- Malignancies, such as lymphoma
- Allergic disease, such as atopic dermatitis, food allergy, and allergic rhinosinusitis and asthma

Before initiating immunologic testing, the clinician should perform a thorough clinical history and physical examination. In infants and children, height and weight records should be reviewed, as failure to thrive and poor growth are consistent with immunodeficiency. In patients with possible immunodeficiency, important elements of the clinical presentation include:

- The nature of the infections: This should include the frequency, chronicity, severity, and response to therapy. Other considerations include what organ system(s) is involved and what type of organism has been identified in the past (ie, viral, bacterial, fungal, opportunistic), since patterns of infections can suggest specific immune defects
- Age of onset of illness, since different immune problems present in infancy, childhood, and adulthood
- The patient's sex, because X-linked defects are mostly or exclusively seen in boys
- Any associated nonimmunologic symptoms and signs, as revealed by a complete review of systems

Initial screening laboratory tests. In patients of any age, the laboratory evaluation of the immune system begins with general studies, including:

- Complete blood count with differential: Lymphopenia is characteristic of a variety of combined cellular and antibody deficiency immunodeficiencies. Lymphopenia is defined as an absolute lymphocyte count <1500 cells/microliter in adults or <2500 cells/microliter in infants. Neutropenia can be found in primary phagocyte disorders, as well as in neutrophil disorders that lead to secondary immunodeficiency. Leukocytosis is sometimes noted and suggests chronic infection.
- Chemistry panels to assess for metabolic disorders (diabetes mellitus, renal disease) that might cause secondary immune deficiency. Hypoalbuminemia or low serum proteins suggest malnutrition or protein loss. Markedly elevated globulin levels may be seen in gammopathies or chronic infections.
- Urinalysis for proteinuria, casts, or cells, which suggest nephritis.
- Tests to evaluate for specific infections, if indicated by the presentation (eg, appropriate cultures, chest and/or sinus imaging): Sinus films may uncover extensive chronic sinusitis in patients with immune deficiency. Children or adolescents with nasal polyposis (although not adults) should be

evaluated for cystic fibrosis, which is a cause of frequent sinopulmonary infections. Chest radiographs of an infant showing absence of a thymic shadow should prompt an emergent evaluation for severe forms of immunodeficiency. In older children and adults, chest radiographs may show scarring from past infections, interstitial lung disease, or bronchiectasis. Hyperinflated lung fields suggest chronic obstructive lung disease or chronic asthma.

- Erythrocyte sedimentation rate and/or C-reactive protein: Nonspecific elevations in acute phase reactants can be seen with infectious and inflammatory disorders and suggest the need for further evaluation.

Referral. More advanced immunologic tests require varying degrees of expertise to perform and interpret, may not be widely available, and are often costly. In addition, knowledge about the possible diagnoses in question is invaluable in deciding the type of testing to pursue first. Thus, immunologic testing is best performed in a graded fashion, and referral to an allergist/immunologist should be sought early in the process, when possible.

Ultimately, definitive diagnosis may require specialized flow cytometric or molecular methods available in reference laboratories.

CATEGORIES AND PREVALENCE OF IMMUNE DEFICIENCIES — Immune deficiency disorders may be grouped into categories based upon the part of the immune system that is predominantly affected. The prevalence of each group of disorders in the population has been estimated to include:

- Antibody deficiencies and defects account for approximately **65 percent** of identified immunodeficiency disorders
- Combined antibody and cellular deficiencies: **15 percent**
- Disorders of phagocytes: **10 percent**
- Isolated cellular defects: **5 percent**
- Disorders of the complement system: **5 percent**
- Other disorders of innate immunity: **<1 percent**

Each of these categories of disorders has characteristic clinical manifestations, although there are varying degrees of overlap among the clinical presentations of the groups.

OVERVIEW OF CLINICAL MANIFESTATIONS AND TESTING FOR SPECIFIC COMPONENTS OF THE IMMUNE SYSTEM

Antibody deficiency and defects.

Antibody deficiency most frequently results in recurrent and severe sinopulmonary infections with encapsulated bacterial strains (eg, Streptococcus pneumoniae, Haemophilus influenzae). Children commonly present with recurrent otitis media, sinusitis, and pneumonia. Adults present similarly, although otitis media is less common. Viral infections of the respiratory tract also occur with greater frequency and severity in these patients. Clinical presentation is discussed in more detail elsewhere.

The age of the patient can help narrow the differential diagnosis:

- The most common antibody defects that present in **infancy** are transient hypogammaglobulinemia of infancy, selective antibody deficiency (after the age of two years) and selective IgA deficiency.
- The most common antibody deficiencies in **young children** are selective antibody deficiency, selective IgA deficiency, IgG subclass deficiency, and early-onset common variable immunodeficiency.

- The most common disorders that present in **adulthood** include common variable immunodeficiency, selective IgA deficiency, IgG subclass deficiency, and selective antibody deficiency.

Measurement of antibody levels. Measurement of immunoglobulin G (IgG), IgA, and IgM is useful in all cases of suspected antibody deficiency.

There are several methods for determining serum Ig levels, and laboratories use different systems. Therefore, it is critical that age-adjusted normal reference ranges are provided. Hypogammaglobulinemia is defined as an IgG less than two standard deviations from normal, and agammaglobulinemia is usually considered when IgG is <100 mg/dL. Panhypogammaglobulinemia (ie, low levels of IgA, IgG, and IgM) is a hallmark of most forms of severe combined immune deficiency. In other combined immune deficiencies, as well as in several predominantly humoral immune deficiencies, there are characteristic alterations in the profile of immunoglobulin (Ig) isotypes that may aid in diagnosis (eg, selective IgA deficiency, selective IgM deficiency, and hyper IgM immunodeficiencies).

In addition to IgG, IgA, and IgM, measurement of IgG subclasses and IgE may be useful:

- Measurement of IgG subclasses may be valuable in children older than one year of age and in adults, particularly if the IgG level is borderline low and/or there is a weak or absent antibody response to vaccination.
- Measurement of IgE is helpful in patients with recurrent sinopulmonary infections, as an elevation is consistent with underlying allergic disease. A very elevated IgE (ie, >2000 IU/mL) in a patient with recurrent bacterial infections and dermatitis would raise suspicion for hyperimmunoglobulin E syndrome.

Measurement of serum IgD is **not** useful for the diagnosis of immune deficiency.

Measurement of antibody function. Antibody function can be assessed by measuring antibody titers (usually IgG) to specific organisms (aka specific antibody) in response to intentional immunization or natural infection. Antibody function is critical for determining the integrity of humoral immunity for two reasons:

- Clinically significant impairment in antibody function can be present even when serum antibody levels are normal.
- Normal responses to immunization (ie, normal antibody function) can occur with subnormal levels of total IgG or of IgG subclasses. This pattern is typically seen with secondary causes of hypogammaglobulinemia, such as that caused by medications, protein loss, or severe malnutrition.

Antibody function is assessed by examining the patient's response to the two general types of antigens; protein antigens and polysaccharide antigens. Routine vaccinations provide examples of both types:

- Measurement of antibody titers to tetanus, diphtheria, Hemophilus influenzae B, and protein-conjugated pneumococcal vaccines (eg, Prevnar) are used to assess response to protein antigens. Antibody titers to other vaccines (eg, hepatitis A and hepatitis B, measles, others) can also be used.
- Measurement of antibody titers to multiple serotypes in pneumococcal polysaccharide vaccines are used to assess response to polysaccharide antigens. This evaluation is useful in adults and children older than two years. This response is particularly important in making a diagnosis of selective antibody deficiency.
- Isohemagglutinins are antibodies generated in response to polysaccharides of gut flora and which cross-react with A or B blood group erythrocyte antigens. They generally appear in the blood by six months of age in individuals who have blood types other than AB. However, vaccine

responsiveness is generally considered to be a more reliable indicator of intact humoral immune function. Isohemagglutinin testing is reviewed in more detail separately.

Assessing vaccine responses is reviewed in detail separately.

Measurement of immune globulin loss. Low levels of immunoglobulins are occasionally due to loss of immune globulin into the GI tract, urine, lung, skin, or peritoneal fluid during dialysis in patients with certain chronic diseases. A method of evaluating the rate of IgG loss is reviewed separately.

Defects in cellular immunity.

Specific cellular immunity is mediated by T cells, and defects affecting these T cells underlie the most severe immune deficiencies. However, because antibody production by B cells requires intact T cell function, most T cell defects lead to combined (cellular and humoral) immune deficiency.

An evaluation of cellular immunity is appropriate for patients with severe viral and/or bacterial illnesses or opportunistic infections. The disorders that can impair cellular immunity are different in various age groups:

- In children younger than one year of age, primary immunodeficiencies are the most common cause of impaired cellular immunity, although perinatal cytomegalovirus and other herpes virus infections can cause transient or persistent cellular immunodeficiency.
- In older children and adults, the major causes are HIV infection and iatrogenic immune suppression due to therapy for autoimmune disease, malignancy, or transplantation. Occasionally, mild forms of primary combined immune deficiency or DiGeorge syndrome escape diagnosis until adolescence or adulthood.

The most profound combined immunodeficiencies are classified under the heading “severe combined immunodeficiency” or SCID. SCID disorders usually present in infancy, while less severe combined immunodeficiencies present in children and occasionally, in adolescents or adults.

Complete blood count with differential and blood smear. The complete blood count (CBC) with differential and blood smear provides valuable information about the cellular immune system. Normal lymphocyte counts in infants are much higher than in older children and adults. In many primary immunodeficiency disorders, cell populations are initially normal, and then decline over time. Thus, normal results in the past cannot be relied upon as a reflection of the current state.

In patients suspected of having a defect in cellular immunity, the CBC establishes the presence or absence of lymphopenia and any associated gross hematologic abnormalities, some of which may greatly assist in diagnosis. A single finding of lymphopenia should be interpreted with caution, since transient lymphopenia is frequently found in a variety of common infectious illnesses. However, significant lymphopenia that does not rapidly correct should not be ignored, since lymphopenia may be the first indication of cellular immunodeficiency or another serious disease (eg, lymphoma) (table 2. 1). In rare situations, a normal lymphocyte count can be seen in the presence of a severe immunodeficiency. If the clinical presentation is highly suggestive of an underlying disorder (eg, a patient with Pneumocystis pneumonia and invasive candida), then lymphocyte subsets should be evaluated with flow cytometry even if the total lymphocyte count is normal.

Evaluation of lymphopenia. Lymphopenia can be caused by an array of disorders. Flow cytometry to evaluate lymphocyte populations should be performed in all patients suspected of cellular immunodeficiency with any significant infection and a total lymphocyte count <1500 cells/microliter (<2500 cells/microliter in infants). In the absence of other indicators of immune dysfunction, there should at the very least be subsequent measurement of the lymphocyte count to document normalization. Persistent lymphopenia requires further investigation of immune function.

Cutaneous delayed-type hypersensitivity. Cutaneous delayed-type hypersensitivity (DTH) is the classic in vivo test of cellular immunity. This test measures the recall response to an intradermal injection

of an antigen to which an individual has already been exposed over a period of time For that reason skin testing is usually not of much value under age two.

Table 2. 1 Causes of lymphocytopenia

Infection	Iatrogenic	Systemic disease	Other causes
1. **Bacterial** (eg, tuberculosis, typhoid fever, histoplasmosis, brucellosis) 2. **Viral** (eg, HIV, severe acute respiratory syndrome [SARS], measles, hepatitis) 3. **Other** (eg, malaria)	1. **Immunosuppressive agents** (eg, glucocorticoids, anti-lymphocyte globulin, alemtuzumab, rituximab) 2. **Chemotherapy** (eg, fludarabine, cladribine), hematopoietic cell transplantation 3. **Radiation therapy** (eg, total body irradiation, radiation injury)	1. **Autoimmune disorders** (eg, systemic lupus erythematosus, rheumatoid arthritis, Sjögren syndrome) **2. Lymphoma** 3. **Other malignancies** **4. Sareoidosis** **5. Renal failure** **6. Aplastic anemia, pancytopenia** **7. Cushing's disease**	**1. Alcohol abuse** **2. Zinc deficiency** **3. Malnutrition, stress, exercise, trauma** **4. Thoracic duct leak, rupture, diversion** 5. **Protein-losing enteropathy**

A positive response to intracutaneous antigen injection requires uptake and processing of antigen by antigen presenting cells, their interaction with CD4+ helper T cells, cytokine production by T cells, and subsequent recruitment and activation of monocytes and macrophages. Thus, skin testing is a sensitive indicator of intact cellular immunity, but negative results must be interpreted with caution, because an impairment in any step of the response pathway will lead to a negative response. In addition, the DTH response is unreliable in children under one year of age (even with prior antigen exposure), and is often suppressed during viral and bacterial infection (even with live attenuated vaccines as in the combined measles, mumps, and rubella vaccine). DTH skin reactivity is also suppressed by antiinflammatory drugs (glucocorticoids) and other immunosuppressants (cyclosporine tacrolimus mycophenolic acid).

Method. Delayed hypersensitivity skin responses can be assessed using an intradermal injection of Candida antigen. This is the only commercially-available reagent intended for use in DTH testing. A 0.1 mL dose of undiluted Candin® is injected intradermally in the volar surface of the forearm. The response is measured 48 to 72 hours after injection. Induration of more than 5 mm is considered positive in all cases. In children, induration of more than 2 mm is sometimes accepted as positive. Erythema alone does not indicate a positive reaction.

In one author’s center (ERS), a panel of four reagents is used for DTH testing: Candin® (as above), Tetanus toxoid (10 flocculation units/mL, Sanofi Pasteur, diluted to 1.5 LF/mL), PPD (5 TU/mL, Sanofi Pasteur), and Trichophyton (1:500 w/v, Allermed Laboratories).

The main advantages of DTH testing are its ease and economy; it is a useful screening test in many instances of suspected cellular immune deficiency. A negative DTH should be followed either with repeated testing (often following a tetanus toxoid booster immunization if tetanus was among the testing reagents), or with measurement of lymphocyte populations by flow cytometry, combined with in vitro

assays of T cell function, as discussed below. In vitro assays (particularly mitogen stimulation) are less sensitive to interference during intercurrent illnesses or by drugs.

Flow cytometry. Flow cytometry uses monoclonal antibodies to identify and quantitate hematopoietic cells that have specific antigens termed cluster designations (CDs). The table lists the fundamental set of markers commonly used and the lymphocyte populations they define (table 2.2). A typical panel of markers used to identify the major subset of lymphocytes includes CD3, CD4, CD8, C19/20, CD16/56/57. The nature and derivation of the nomenclature of the markers are discussed elsewhere.

Table 2.2**Markers commonly used for assessment of lymphocyte subsets by flow cytometry**

Marker name	Cell type	Comment
CD3	T cells	Expressed on all T cells and no other cell type
CD4	T cell subset	Predominantly helper/inducer T cells
CD8	T cell subset	Predominantly cytotoxic T cells; expressed by up to one- third of natural killer cells
CD19 or CD20	B cells	
CD16	Natural killer cells	Some NK cells may not express CD16
CD56	Natural killer cells	Expressed on the majority of NK cells
CD57	Natural killer cells	Expressed on the majority of NK cells; combinations of CD16, CD56 and CD57 will more reliably evaluate NK cell number than any marker alone
CD45RA	Naive T cells	
CD45RO	Memory T cells	

A CBC with differential should be performed on a blood specimen obtained at the time of the flow cytometric analysis, or the cytometer itself be used to determine the lymphocyte number. This analysis permits the calculation of the absolute numbers of each lymphocyte subset. It is possible for the percentage of a particular subset to be abnormal while the total number of cells is within the normal range, and vice-versa. An absolute deficiency, rather than a relative (percentage) deficiency, is of much greater clinical significance.

Flow cytometry is invaluable in the assessment of lymphocyte subpopulations in patients with opportunistic infections or severe or persistent lymphopenia. Standard flow cytometry analysis will be abnormal in almost all cases of severe combined immune deficiency (SCID), and in many instances of other combined immunodeficiencies. The analysis of lymphocyte subsets may be diagnostic for various forms of lymphoma as well. The use of flow cytometry in the diagnosis of specific immunodeficiencies is reviewed in more detail separately.

Abnormalities in immunodeficiency. The table summarizes anticipated alterations in the representations of various lymphocyte populations in several immune deficiency diseases. Specific disorders are reviewed separately.

While certain immune defects are associated with characteristic patterns of lymphocyte subsets, lymphocyte populations may appear to be entirely normal even with clinical evidence of significant immune dysfunction. Conversely, as with total lymphocyte numbers, lymphocyte subsets may be profoundly altered by common infectious illnesses and other factors. Thus, for the purpose of diagnosis, flow cytometry data must be considered in conjunction with functional tests of the immune system.

Decreased numbers or absent natural killer CD16/56 cells (≤100 cells/ul), particularly in the presence of recurrent infection with herpes viruses, should warrant further studies for natural killer cell deficiencies, including cytotoxicity studies.

Advanced tests. Many advanced tests are available to study specific cellular immune defects. These tests are best ordered and interpreted by an immunology expert. Advanced tests include:

- Advanced flow cytometry using monoclonal antibodies specific for activated cells, regulatory T cells, naïve or memory cells, or various stages of B cell development are of value in further characterizing many antibody deficiencies (eg common variable immunodeficiency) or cellular immunodeficiencies (eg immune dysregulation, polyendocrinopathy, enteropathy, X-linked or IPEX syndrome). Flow cytometry can be used in the definitive diagnosis of several genetic immunodeficiencies by assessing the absence or abnormal expression of a specific protein (eg, X-linked agammaglobulinemia, Wiskott-Aldrich syndrome).
- Analysis of T cell receptor excision circles (TRECs) provides an assessment of thymic function by measuring recent thymic emigrant cells. This is also used as a screening test for newborn SCID on newborn blood samples.
- Cytotoxicity assays measure the functional capacity of cytotoxic T cells or natural killer cells. T cell cytotoxicity is a researeh tool since the target cells must share HLA antigens with the patient. One such method utilizes antigenic peptides attached to specific MHC molecules labeled with a fluorochrome as targets. Binding of the cytotoxic cell to the peptide is measured by flow cytometry.
- Cytokine measurements on cells, serum, or tissue sections.
- Mutation analysis to identify mutated genes for many T cell or combined defects.
- Chromosome analysis is of value in the diagnosis of DiGeorge syndrome.
- HLA typing is used to identify chimerism in newborns with SCID or to identify an appropriate stem cell donor.
- CD3/T cell receptor analysis and function.

In vitro studies of T cell function. In vitro studies of T cell function measure peripheral blood T cell proliferation in response to several different types of stimuli:

- Mitogens (such as the plant lectins phytohemagglutinin, concanavalin A, pokeweed mitogen, anti-CD3)
- Specific antigens (such as tetanus and diphtheria toxoids or C. albicans antigens)
- Allogeneic lymphocytes (ie, mixed lymphocyte culture)

These studies may not be possible in patients with profound lymphopenia. In parallel with (or prior to) studies of T cell function, flow cytometric measurement of peripheral blood lymphocyte subpopulations should be performed.

In T cell function studies, the patient's purified peripheral blood mononucleareells (lymphocytes and monocytes) are incubated in sterile media with the test substance(s) or cells for three to six days. A control tube with cells and media alone is also incubated. During the last 24 hours of culture, tritiated thymidine is added to the cultures. Dividing lymphocytes incorporate the thymidine into their DNA. The extent of proliferation is determined by measuring the radioactivity taken up by cells. The test is interpreted as negative, partial, or normal by comparing patient results with normal control lymphocytes assayed simultaneously and with the normal ranges for controls in the laboratory.

Many laboratories also report results as a stimulation index (SI). This is the ratio of radioactivity (as counts per minute, or CPM) with stimulation over the CPM in the control tube without stimulation (background).

Suppression of T cell responses to mitogens and antigens may be seen with significant nutritional deficiencies, moderate to severe concurrent illnesses, or with administration of immunosuppressive drugs. Thus, these tests should be performed when the patient is relatively well and not receiving glucocorticoids or other immunosuppressive medications, whenever possible. T cell responses appear to normalize rapidly (ie, within a day or two) after glucocorticoids are discontinued.

Response to mitogens. Most mitogens require functional antigen presenting cells (ie, monocytes and B cells) for T cell stimulation, although the mechanisms of antigen processing and presentation are bypassed. Mitogens are powerful stimulants for T cells, and responses may be assessed in patients of any age, even newborns, regardless of immunization status.

Normal reference ranges are derived from studies on the cells of healthy adults. Newborns generally have higher mitogen responses compared to adults. Depending upon the mitogens and the laboratory, the range of counts per minute (CPM) reported for normal controls is approximately 50,000 to 300,000 and SI between 10 and 200. Phytohemagglutinin (PHA) is most commonly used. Results for patients in comparison to controls are roughly interpreted as normal (>50 percent of control), low (25 to 50 percent), very low (10 to 25 percent), or absent (<10 percent). Results expressed as SI vary widely between laboratories. In general, a SI <10 may be considered as no response.

Some researehers have also used in vitro stimulation with the OKT3 monoclonal antibody as a measure of T cell function. OKT3 binds to the epsilon chain of the T cell CD3 complex and stimulates T cells (in the presence of accessory or antigen presenting cells) in a manner analogous to T cell mitogens. Cell response to mitogen or OKT3 is generally similar, although they may sometimes give different patterns in the context of distinct immune defects. There are no published comparisons in a large group of normal individuals or immune deficient patients. Most clinical reference laboratories use one or more mitogens and one or more antigens, but do not routinely use OKT3.

Diminished or absent proliferation signals a serious derangement of T cell function. Mitogen responses will be severely depressed (usually well below the control fifth percentile) in the vast majority of patients with profound lymphopenia (eg, complete DiGeorge syndrome), severe combined immune deficiency (SCID), or advanced AIDS. By comparison, the response may be partial or normal in milder syndromes of combined deficiency (eg, Wiskott-Aldrich syndrome), in mainly humoral deficiency, or in those with infections (especially CMV and other herpes viruses).

Response to specific antigens. Specific antigen tests are more sensitive than mitogen assays as indicators of defects in T cell function, since the mechanisms of activation are more complex than those for mitogens. These tests require antigen processing and presentation by antigen presenting cells, although T cell recruitment of effector cells (eg, in vivo DTH) is not necessary. As with DTH, in vitro specific antigen proliferation tests are not useful in infants or other patients who have not completed a primary series of immunizations.

Tetanus and diphtheria toxoids and monilia (Candida) are antigens frequently used for this assay. Since only a small number of cells respond, the cultures must be maintained longer, and incorporation of radioactivity is lower than for mitogen stimulation. Results expressed as CPM are often reported from 5000 to 50,000; results as SI generally range from 43 to 50. Because of the very large variation in the frequency of antigen-specific T cells in the blood of healthy controls, the SI is often most important for interpretation. In general, a SI for specific antigen >43 is considered adequate.

In many cases of combined immune deficiency, responses to specific antigens are diminished or even absent while mitogen responses remain intact. In secondary immune deficiency, specific antigen responses will also generally be suppressed more easily than mitogen responses. However, these

generalizations are not always true. As with DTH testing, booster immunization with tetanus followed by a repeat in vitro proliferation assay may detect a significant response.

Another way to assess function of T cells is to measure cytokine release following antigen exposure. An example of this type of test that has become widely used in the diagnosis of latent tuberculosis infection is the interferon release assay, which can be used instead of tuberculin testing.

Neutrophil defects (defects in phagocyte function). Neutrophil defects result in a range of illness from mild recurrent skin infections to overwhelming, fatal systemic infection. Affected patients are more susceptible to bacterial and fungal infections, but have a normal resistance to viral infections. Most are diagnosed in infancy due to the severity of the infection or the unusual presentation of the organism, but some escape diagnosis until adulthood.

Primary phagocytic deficiencies characteristically lead to recurrent and severe fungal (eg, Candida and Aspergillus) and bacterial (eg, Staphylococcus aureus, Pseudomonas aeruginosa, Nocardia asteroides, Salmonella typhi) infections. Response to nontuberculous mycobacteria may also be abnormal, particularly in patients with chronic granulomatous disease. The most common sites of infection are the respiratory tract and skin. Tissue and organ abscesses also occur. Other frequent manifestations include abnormal wound healing, dermatitis/eczema, stomatitis, and delayed umbilical cord detachment. Many patients have growth failure.

Phagocytic disorders can be caused by extrinsic or intrinsic defects. Extrinsic defects include opsonic abnormalities secondary to deficiencies of antibody and complement factors, suppression of granulocyte production, and leukocyte autoantibodies or isoantibodies that decrease the number of circulating neutrophils. Intrinsic disorders of granulocytes may be divided into defects in granulocyte killing ability and defects in chemotaxis (cell movement).

Evaluation of neutrophil numbers. The initial test for evaluation of phagocyte disorders in the CBC with differential:

- Leukocytosis raises the possibility of a leukocyte adhesion defect (ie, a defect in chemotaxis), and flow cytometry is indicated next to assess for the presence of specific adhesion molecules.
- Severe neutropenia can be seen with congenital agranulocytosis or cyclic neutropenia.
- If neutrophil numbers are normal and the patient's history is consistent with a neutrophil disorder, then an evaluation of neutrophil function is appropriate.

Evaluation of neutrophil function. Disorders of neutrophil function include chronic granulomatous disease (CGD), Chediak-Higashi syndrome, neutrophil-specific granule deficiency, leukocyte adhesion defects, and hyper IgE syndrome. Initial tests include:

- Neutrophil oxidative burst is best measured by a flow cytometric assay of dihydrorhodamine (DHR). This test is quantitative and is useful in the identification of heterozygote carriers of CGD. This procedure is widely available in reference laboratories and has generally replaced the nitroblue tetrazolium test (NBT test). However, the NBT test can be used for screening purposes.
- An IgE level, which is often elevated in hyper IgE syndrome.
- Examination of a peripheral blood smear to detect:
- The giant azurophilic granules in neutrophils, eosinophils, and other granulocytes, which are diagnostic of Chediak-Higashi syndrome.
- Neutrophils that lack granules and are often bilobed, which are found in neutrophil-specific granule deficiency.
- Advanced mutation analysis and flow cytometric analysis for many neutrophil disorders are also available.
- A chemotaxis assay can be done to identify a cell movement disorder.

Complement defects

Complement disorders may be inherited or acquired. Screening for a classical complement pathway defect is indicated in patients with any of the following:

- Recurrent, unexplained pyogenic infections in whom the white blood count and immunoglobulin levels and specific antibody responses are normal
- Recurrent Neisserial infections at any age
- Multiple family members who have experienced Neisserial infections

In addition, it is reasonable to evaluate the complement system in any patient with systemic lupus erythematosus (SLE). However, this is particularly relevant in those with familial lupus or subacute cutaneous lupus, in whom C1q or C2 deficiency should be excluded.

The initial screening test for complement defects is a total hemolytic complement assay (CH50), which assesses classical pathway function and is widely available. If the CH50 is significantly reduced or zero, then the levels of individual complement components are measured. Low levels of multiple components, particularly low C3 and C4, suggest complement consumption, rather than a primary complement deficiency. If the CH50 is normal and a complement defect is still suspected, the AH50, a screening test for alternative pathway defects, may be obtained. This test is largely performed in specialized laboratories. Alternative complement deficiencies include properdin and factor D deficiencies, both of which are rare. Inherited and acquired defects in complement components are reviewed in more detail elsewhere.

If a patient has frequent infections suggestive of a complement deficiency, a mannose-binding lectin defect should be excluded.

Defects and deficiencies of C1 esterase inhibitor are associated with hereditary angioedema, although these disorders are not immunodeficiencies because these patients do not have increased susceptibility to infection.

Innate immune defects

Defects in innate immune mechanisms are relatively rare disorders that should be suspected in patients in which other immunodeficiencies have been excluded. Many are associated with specific infections (eg, herpes or EBV infections in NK cell defects, atypical mycobacterial infection in IL-12/23-IFN-γ pathway defects, neonatal herpes simplex encephalitis in the TLR-3 signaling pathway and other severe infections, often associated with poor inflammatory response).

Natural killer cell defects — NK cell defect can be primary or secondary. Primary NK deficiency should be suspected in patients with low NK cells and/or recurrent severe herpes virus infections. NK defects are also noted in patients with X-linked lymphoproliferative disorders and Hemophagocytic lymphohistiocytosis, Chediak-Higashi syndrome.

Defects of IL-12/23-IFN-gamma pathways — Patients with invasive infections caused by low virulence Mycobacterial and Salmonella species may have defects of the genetic components of the IL-12/23-IFN-γ pathways including the IFN-γ receptor 1 gene (IFNGR1) or the IFN-γ receptor 2 gene (IFNGR2), the IL-12 receptor β1 gene (IL12RB1), the IL12B gene, and the STAT1 gene. Special tests involving western blotting or flow cytometry can pinpoint the exact molecular defects.

Toll-like receptor pathway defects — Pattern recognition receptors (PRRs) are receptors specific for molecularеomponents of microorganisms widely expressed on phagocytic cells (neutrophils, macrophages, and monocytes and several other cells). Defects in PRRs can cause increased susceptibility to infections that is most severe in infancy, before the adaptive (ie, humoral and cellular) immune system has developed. The frequency and severity of infections generally lessen as the patient matures, in contrast to most other types of immunodeficiency.

The Toll-like receptors (TLRs) are one group of PRRs that, after ligation with specific microorganisms, initiate a series of steps that eventually activate the nucleus to initiate cytokine synthesis.

Several genetic disorders of the signaling pathways have been identified, including an IL-1 receptor-associated kinase 4 defect (IRAK4), a myeloid differentiation primary response gene 88 defect (MYD88), a nuclear factor kappa B essential modulator defect (NEMO), a TLR3 receptor defect, and an UNC-93B defect. Screening tests to evaluate these defects are available, including a flow cytometric assay.

THEME №3. IMMUNE INFLAMMATION AND INFECTIOUS DISEASE. HIV INFECTION: IMMUNOPATHOGENESIS, IMMUNODIAGNOSIS, IMMUNOCORRECTION

We encounter many thousands of microbes every day - many harmless, many beneficial but some that cause disease. The immune system defends us against infections caused by the huge variety of microorganisms we encounter, including viruses, bacteria, fungi and parasites. Microbes divide rapidly, each division allowing genetic variation and change. Thus microbes can change within days or even hours. It takes years for humans to reproduce and generate genomic variation. The immune system has developed elegant mechanisms that facilitate somatic change without genomic variation in response to infection and other stimuli.

THE MAJOR DEFENCE MECHANISMS AGAINST INFECTIONS

Microorganisms come in all shapes and sizes, with some penetrating into cells and others entering the body but remaining outside the cells. Thus the immune system has had to develop several different mechanisms to recognise and kill microbes depending on their characteristics. From the immune systems point of view, microbes can be divided according to type of infection caused.

Extracellular infection

♦Bacteria enter tissues but usually remain outside the cells. However, as they are smaller than cells of the immune system, specialised immune cells can ingest, kill and digest the bacteria.

♦Multi-cellular parasites also remain outside cells, however, as they are larger than immune cells, they cannot be ingested and so additional immune mechanisms are required to fight infection.

Intracellular infection

♦Viruses enter the cytoplasm, hijack the host cells protein synthesis machinery and assemble new virus particles, which bud from the cell surface and infect new cells. Immune mechanisms which act in the extracellular space are ineffective once virus enters the cells.

♦Intra-vesicular organisms (e.g. Mycobacteria) are taken up into cells but remain within vesicles, never entering the cytoplasm. Immune mechanisms that kill virus-infected cells are ineffective as the organisms are in a different cell compartment - therefore requiring an additional immune strategy.

Infecting organisms must first breach the body's natural defences (skin, mucous membranes etc.). The pathogen then faces the two major types of immune response, the innate immune response, and the adaptive or specific immune response. When thinking about how these systems work it is helpful to consider (1) the recognition phase where the micro- organism/pathogen is recognised as foreign, and (2) the effector phase, which kills the organism.

The innate immune response is immediately available to fight pathogens without the requirement for prior exposure to the pathogen. This is the first line of defence against pathogens, recognising microbes by the presence of molecular patterns not present on mammalian cells. Innate immunity is moderately effective at controlling infection and does not improve with repeated exposure to a particular organism.

The adaptive immune response is refined and expanded after infection, taking several days to provide protection on first exposure to a particular pathogen. The cells and molecules produced are highly specific for the pathogen. The adaptive immune system remembers when a microbe has previously invaded the body resulting in a rapid and efficient removal of the pathogen on the second and third time that it invades the body (immunological memory).

Innate and adaptive immunity depend on white blood cells or leucocytes. Innate immunity involves granulocytes and macrophages. Adaptive immune responses depend on lymphocytes, which provide the lifelong immunity that can follow exposure to disease or vaccination. However, the two systems do not operate independently - there are many examples of co-operation. Killing of microorganisms by the adaptive immune response frequently depends upon linking antigen-specific recognition to activation of effector mechanisms that are also used in the innate response.

Together, the innate and adaptive immune systems provide an amazing defence system. Despite the fact that we are surrounded by a multitude of potentially pathogenic microorganisms, we rarely succumb to infection. Many infections are eliminated by the innate immune system and cause no disease. Infections that cannot be resolved by innate immunity trigger adaptive immunity, which usually eliminates the infection (often before we are aware of it) and generates immunological memory.

THE DEFENCE SYSTEMS OF THE BODY FALL INTO THREE CATEGORIES

♦Non-immunological external defences

♦Innate immunity

♦Adaptive immunity.

The principle difference between innate and adaptive immune responses is in pathogen recognition. Many effector mechanisms used to kill pathogens are shared by both systems.

NON-IMMUNOLOGICAL EXTERNAL DEFENCES Skin and mucus membranes form physical barriers to infectious organisms. When breached (e.g. burn victims) infection is common despite normal immune function. Pathogens must attach to epithelial cells and migrate through the epithelium to establish infection. Surface epithelia provide mechanical, chemical and microbiological barriers as a first line of defence against infection (Figure 3.1).

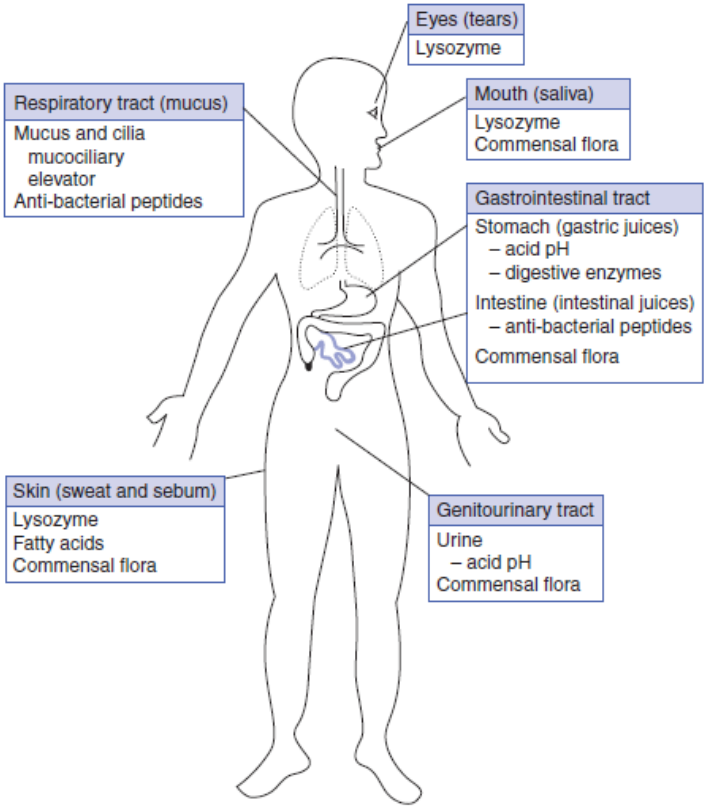

Figure 3.1 External defences of the body.

Mechanical barriers prevent microbial attachment and include

♦Flow of secretions across epithelium - when flow is obstructed infection is common.

♦Mucociliary elevator. Inhaled organisms are trapped by mucus, which is moved by the coordinated action of cilia on the surface of ciliated epithelium. In the respiratory tract, mucus and organisms are moved to the oropharynx and swallowed or expectorated.

Chemical barriers Chemical barriers include the acid environment in the stomach, together with digestive enzymes and the antimicrobial effect of lysozyme found in tears and sweat, as well as defensins (bactericidal peptides found in the respiratory and gastrointestinal tracts).

Microbial barrier Commensal flora, found on skin and mucosal membranes, form a microbial barrier by competing for nutrients and attachment sites on cells. Some also produce anti-bacterial substances.

INNATE IMMUNE RESPONSES When microorganisms penetrate epithelial surfaces they are usually killed by the innate immune response (Figure 3.2). Innate immunity acts immediately, recognises broad microbial patterns rather than unique specificities and does not produce immunological memory.

Macrophages and neutrophils have surface receptors that recognise and bind common constituents of many bacteria. Binding induces engulfment, killing and degradation of bacteria - termed phagocytosis.

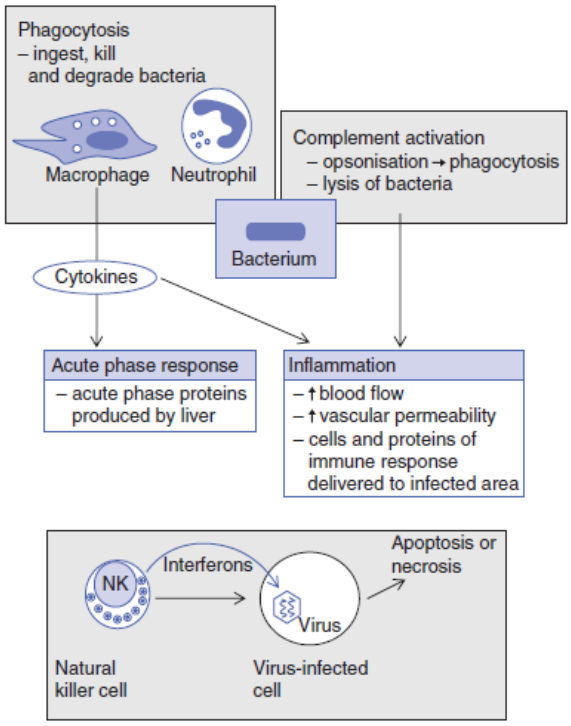

Figure 3.2 The innate immune response: overview.

Following phagocytosis, activated macrophages secrete chemical messengers (cytokines), which initiate inflammation. Inflammation increases blood vessel permeability, rapidly increasing delivery of cells and proteins of the immune system to the infected area. Activation of complement, a system of plasma proteins, generates fragments that coat or opsonise bacteria increasing the efficiency of phagocytes. Complement also lyses some bacteria, and releases small pro-inflammatory peptides.

Macrophage activation results in cytokine release, causing a rise in body temperature and an acute phase response. Acute phase proteins are produced in the liver and contribute to inflammation and host defence.

NK cells recognise and kill virus-infected cells. NK cells are activated by cytokines (TNF-α. and IL-12) produced by macrophages. Virus infected cells produce interferon-α and β (IFN-α. and β), which inhibit viral replication within cells, make surrounding cells more resistant to viral entry, and also activate NK cells.

The innate immune response makes a crucial contribution to activation of adaptive immunity

♦Macrophages enhance the adaptive immune response by acting as antigen presenting cells (APCs).

♦Cytokines produced by cells (macrophages and NK cells) of innate immunity enhance responses by the adaptive immune response.

♦The inflammatory response increases the flow of lymph containing antigen and APCs to the lymphoid tissue.

ADAPTIVE IMMUNE RESPONSES Adaptive immunity provides protection when innate immunity fails to eliminate infection. Adaptive immunity develops slowly, has unique specificity for antigen and produces immunological memory (Figure 3.3).

Adaptive immunity results in selection of lymphocyte clones bearing highly antigen-specific receptors (recognition molecules). Each lymphocyte expresses cell-surface receptors of a single specificity. B cell receptors (immunoglobulins) bind extracellular molecules and pathogens, while T cell receptors (TCR) bind peptide fragments bound to MHC molecules on cell surfaces.

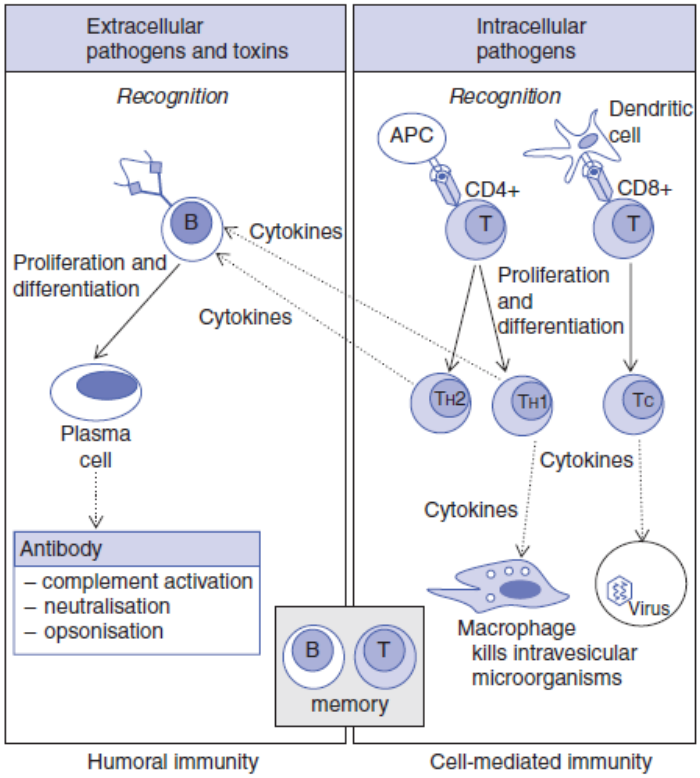

Figure 3.3 The adaptive immune response: overview.

Following initiation of an immune response, the antigen-specific lymphocyte(s) proliferate and its progeny differentiate into effector cells that can eliminate the pathogen. A subset of these proliferating lymphocytes differentiates into memory cells capable of responding rapidly if the same pathogen is encountered again.

Adaptive immunity can be divided into humoral (antibody-mediated) immunity, and cell-mediated immunity. Both types of immune response require activation of helper T cells (TH), a pivotal lymphocyte subset that are essential for the development of effective adaptive immune responses (Table 3.1).

ANTIGENS

The term antigen originally described any substance that could induce antibody formation. Antigens include proteins, carbohydrates, lipids and nucleic acids. Following characterisation of the cellular immune response, the use of the term antigen was expanded to include any substance that activated the immune response. Autoantigens are molecules found on host tissues that can induce an immune response.

Table 3.1 Features of innate and adaptive immunity

CHARACTERISTICS	CELLS	MOLECULES
Innate immunity		
Rapid response within hours	Macrophages	Cytokines
No unique specificity	Neutrophils	Complement
No memory	NK cells	Acute phase proteins
		Natural opsonins
Adaptive immunity		
Slower reponse ~5-6days	T and B lymphocytes	Antibodies
Highly specific	Plasma cells	Cytokines
Memory		

Antigenic determinants and epitopes Antibodies or lymphocytes produced in response to an antigen are directed against specific parts of the molecule called antigenic determinants or epitopes and not against the whole molecule. Epitopes are the smallest unit of antigens which can elicit an immune response. One molecule may have several identical or different epitopes, for example, a carbohydrate with repeating sugar units has several identical epitopes, while a large single chain protein has many different antigenic epitopes.

Conformational and linear epitopes Antibodies usually bind conformational epitopes dependent on folding of the molecule (tertiary structure). Molecular folding brings different parts of a linear peptide chain close together, forming a single, discontinuous epitope. Many antibodies bind conformational epitopes in native proteins, but will not bind the denatured unfolded molecule. In contrast, T cell receptors recognise linear epitopes arising from the linear amino acid sequences of peptide antigens (primary structure) (Figure 3.4).

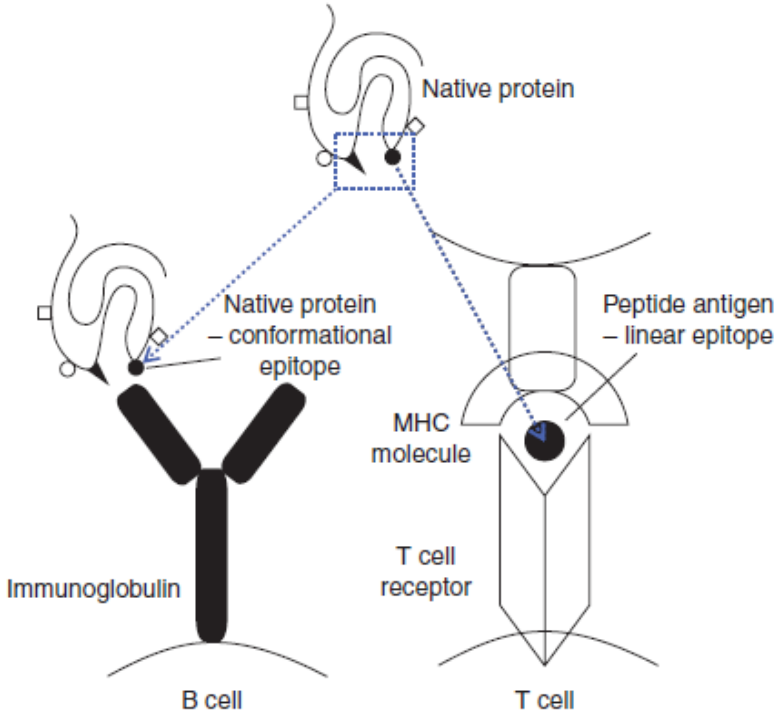

Figure 3.4 T and B cell epitopes.

Haptens Haptens are small molecules that can bind antibody but are incapable of inducing an adaptive immune response alone. Haptens must bind carrier molecules to elicit antibody and T cell responses. Haptens such as drugs may elicit responses when bound to host proteins and anti-hapten antibodies may mediate allergic drug reactions.

IMMUNOGENICITY Molecules that can stimulate an adaptive immune response are said to be immunogenic and can also be called immunogens. Factors other than antigen characteristics influence immunogenicity (Table 3.2). Substances that improve an immune response to antigens are called adjuvants. Purified proteins may be poorly immunogenic and carbohydrates, nucleic acids and other types of molecule usually require modification to induce an immune response.

Table 3.2 Factors that influence the immune response to an antigen

Nature of molecule	Protein content Size Solubility *Large, complex, particulate, denatured proteins are most effective*
Dose	Low dose - high antibody affinity + restricted specificity Moderate dose - varying affinity antibodies + broad specificity High dose - tolerance *Moderate dose more immunogenic*
Route of entry	Oral - Peyer's patches Inhalation - bronchial lymphoid tissue IV - spleen ID, IM, SC - regional lymph nodes *Subcutaneous injection induces strongest responses*
Substances with synergistic effects	Adjuvants Alum used in many human vaccines *Bacterial products may provide stronger adjuvants in the future*

Note: ID - intradermal; IM - intramuscular; SC - subcutaneous; IV – intravenous

TARGETS OF THE INNATE IMMUNE RESPONSE Groups of organisms often have distinctive molecular patterns that are targets of the innate immune response. Table 3.3 summarises the most important of these targets and corresponding organisms, the pattern recognition receptor or molecule and their cellular location. Pattern recognition molecules (PRMs) are utilised by the innate immune response, to facilitate removal of pathogens.

Table 3.3 Molecular targets of PRMs of the innate immune system

INNATE TARGETS	RECOGNITION MOLECULE: PATTERN RECOGNITION RECEPTOR	CELLULAR LOCATION
Mannosyl/fucosyl structures *Pseudomonas aeruginosa Mycobacterium tuberculosis Candida albicans Pneumocytsis carinii Klebsiella pneumoniae Leishmania donovani*	Mannose receptor	Macrophages Endothelial cells Dendritic cells
LPS, Gram-negative bacteria *E. coli* *Neisseria* *Salmonella*	CD14 LPS-binding protein (LBP), an essential adaptor protein	Macrophages
Carbohydrates or lipids Bacterial and yeast cell walls	Scavenger receptor	Macrophages
LPS Gram-negative bacteria	TLRs	APCs B cells Macrophages
Peptidoglycans, teichoic acids Gram-positive bacteria Arabinomannans, glucans		

Antigen targets of the adaptive immune response: thymus-dependent and thymus-independent antigens

Thymus-dependent (TD) antigens (most proteins) require T cell help to induce antibody production. Thymus-independent (TI) antigens induce antibody production without T cell help; some bacterial polysaccharides, polymeric proteins and lipopolysaccharides can directly stimulate specific B cells. B cell responses to TI antigens are particularly important in organisms whose surface antigens elicit weak peptide-specific T cell responses. In children adequate responses to Tl-antigens can take 4-6 years to develop (Table 3.4).

Table 3.4 Thymus dependent and thymus independent antigens.

TD-ANTIGEN	TI-1 ANTIGEN	TI-2 ANTIGEN
Diptheria toxin Viral haem agglutinin Purified proteins derivative (PPD) of M. tuberculosis	Bacterial lipopolysaccharide Brucella abortus	Pneumococcal polysaccharide Salmonella polymerised flagellin Dextran Hapten-conjugated Ficoll (polysucrose) Haemophilus influenza capsular polysaccharides

There are two types of Tl-antigens that activate B cells by different mechanisms:

♦TI-1 antigens possess intrinsic B cell stimulatory activity. At high concentrations TI-1 antigens activate the majority of B cells independently of their specific B cell receptor, acting as mitogens. At low concentrations, only B cells specific for the TI-1 antigen are activated. Bacterial lipopolysaccharides (LPS) are TI-1 antigens.

♦TI-2 antigens are linear molecules with highly repetitive structures, for example, bacterial capsular polysaccharides. TI-2 antigens activate mature B cells by extensive cross-linking of their specific B cell receptors. The capsular polysaccharide of Haemophilus influenzae type B is a TI-2 antigen.

Lymphocyte Maturation Lymphocytes must recognise a huge diversity of antigens and must develop the functional capacity to play many roles in generating, enhancing and regulating specific immune responses. Each lymphocyte expresses a single specificity of antigen receptor. B and T cell maturation pathways share many common features but anatomical sites of maturation, and some molecular events in antigen receptor generation are distinct to each population. NK cells are another population of lymphocytes, which do not interact with antigens in a specific manner, and do not express rearranged antigen specific receptors.

The functions of lymphocytes are:

♦Antibody production (specifically B cells)

♦Cell-mediated inflammation (T cells)

♦Regulation of other immune cell functions (T cell)

♦Cytotoxicity (T cell and NK cells).

ANTIGEN (HLA) MOLECULES

HLA molecules are the human MHC molecules. HLA molecules play a pivotal role in the normal immune response, and inability to express these molecules causes a lethal immunodeficiency. An individual's HLA type determines in part their ability to respond to infection and predisposition to autoimmune disease. Matching donor and recipient HLA types as closely as possible improves outcome in most types of transplantation.

HLA MOLECULES - STRUCTURE AND FUNCTION HLA molecules are divided into two main classes, HLA Class I (including HLA-A, B and C) and HLA Class II molecules (including HLA-DR, DP and DQ). HLA Class I molecules have one variable a chain, and are stabilised by B2-microglobulin.

HLA Class II molecules are heterodimers, comprising 2 variable chains (a and β chains). Both Class I and Class II molecules have a peptide-binding groove (Figure 3.5). The physiological function of both classes of HLA molecules is to present short pathogen-derived peptides to T cells. This is the key interaction in initiating an antigen specific, adaptive immune response.

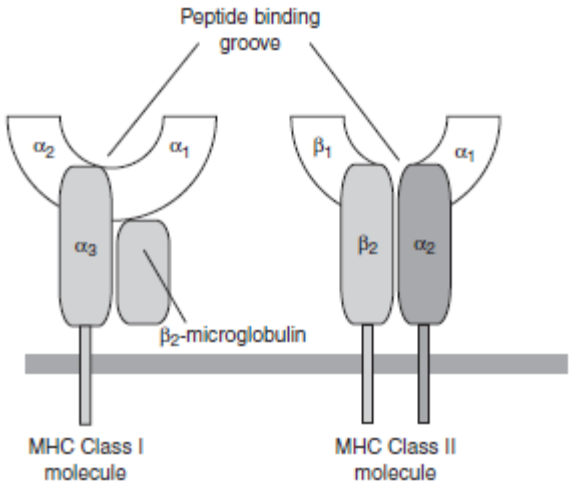

Figure 3.5 Structure of MHC (or HLA) Class I and Class II molecules.

HLA molecules bind many different peptides stably as an integral part of the HLA molecule - HLA is unstable when peptides are not bound. The peptide binding domains of HLA Class I, and HLA Class II molecules are polymorphic; that is, particular amino acids in the peptide-binding grooves vary from allele to allele. These polymorphic residues make contact with the antigen-derived peptide. The peptide amino acids that bind HLA are called anchor residues. Peptides range in length from 8 to 10 residues for HLA Class I, to 12-20 residues for HLA Class II.

HLA Class I molecules are expressed on all nucleated cells and on platelets and present cytoplasmic peptides to CD8+ cytotoxic T cells. Widespread expression of HLA Class I molecules allows all cells to present peptides from intracellular pathogens to cytotoxic T cells of the immune system.

HLA Class II molecules are expressed on a restricted repertoire of immune cells including dendritic cells, monocytes and macrophages, B cells, activated T cells and thymic epithelial cells. HLA Class II molecules present antigen to CD4+ helper T cells to initiate adaptive immune responses, a role requiring restricted expression. At sites of inflammation, particularly in the presence of IFN-γ, Class II molecules are induced on cells that do not normally express these molecules.

The genes encoding the HLA molecules are found in a small cluster on Chromosome 6 (Figure 3.6). This region contains in excess of 200 genes including both HLA, non-HLA genes involved in the immune response, and many genes with no apparent immunological role.

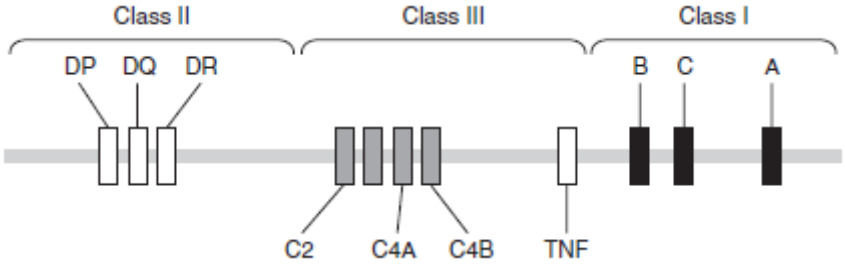

Figure 3.6 Chromosome 6: genes in the Major Histocompatibility region encode HLA molecules.

HLA MOLECULES - DIVERSITY Within the population, there are hundreds of different HLA alleles. Most variability between different alleles of a HLA molecule resides around the peptide-binding groove. Expression of HLA molecules is polygenic (we each have several HLA genes) and polymorphic (at each HLA gene locus there are many possible alleles). Individuals of differing HLA type infected with the same organism will usually present different pathogen-derived peptides to initiate an immune response. The outcome of the immune response is often equivalent, however, possession of particular HLA types has been associated with disease resistance in some situations. For example, expression of HLA-B53 is associated with reduced mortality from severe malaria.

We inherit one set of HLA genes from each parent, and both maternal and paternal alleles are expressed on all cells. Within particular populations gene 'packages' known as haplotypes are frequently identified. Thus some HLA genes may be found almost exclusively associated with typical haplotypes within a particular population. These haplotypes include HLA genes and also the intervening genes encoded on chromosome 6, many of which have immunological functions. Haplotype associations of HLA molecules differ in different populations.

Genetic diversity of HLA molecules helps to ensure that some members of a population can mount an immune response to any organism. While some individuals may be unable to eliminate a pathogen, diversity makes survival at population levels more likely. At an individual level, people who are heterozygous (expressing different maternal and paternal HLA alleles) can bind and present a greater repertoire of peptides to their T cells. It appears that progression to acquired immunodeficiency syndrome (AIDS) is slower in human immunodeficiency virus (HlV)-infected individuals who are heterozygous for HLA molecules, suggesting a possible survival advantage attributable to HLA diversity.

DISEASE ASSOCIATIONS Particular HLA types have been associated with both disease susceptibility and resistance. Associations are relative rather than absolute, and therefore are rarely useful in establishing a diagnosis in individual patients. Apparent associations may reflect a true role of the HLA molecule or may be due to linkage between the HLA allele and a physically close disease related (non-HLA) gene. In the non-immunological sleep disorder narcolepsy, an apparent HLA association was explained by linkage disequilibrium between a mutated gene encoding a hypocretin receptor and particular HLA alleles (Table 3.5).

Table 3.5 HLA and disease associations.

DISEASE	HLA ASSOCIATION	RELATIVE RISK
Ankylosing spondylitis	B27	87.4
Rheumatoid disease	DR4	4.2
Diabetes (Type I)	DR4	6.4
Diabetes (Type I)	DR2	0.19
Dermatitis herpetiformis	DR3	15.9

***Note**: Relative risk is the risk of disease in individuals expressing specific HLA type, divided by the risk in those who do no express this HLA antigen.*

Several non-HLA genes are encoded in the HLA region of chromosome 6, many of which affect the immune response. For example, the TNF-a gene is encoded in this region, and a functional polymorphism in this gene determines whether large or small amounts of TNF-a are produced. The high-producing TNF-a genotype is linked to HLA-DR3 and included in a haplotype associated with increased risk of autoimmunity. Determining whether an apparent HLA association is significant is complicated by the common occurrence of haplotypes. Demonstrating a true HLA effect usually requires the study of several populations where individual HLA molecules are found with different haplotype associations.

ANTIGEN PRESENTATION

The protective function of T cells depends on their ability to detect cells that harbour or internalise pathogens. T cells do this by responding to pathogen-derived peptides bound to MHC molecules on cell surfaces. Antigens may be endogenous (derived from molecules inside the cell, including molecules derived from intracellular pathogens) or exogenous (occurring outside the cell, but taken up by the processing cell). The generation of peptides from antigens is known as antigen processing. Not surprisingly, different mechanisms are involved in processing endogenous and exogenous antigens. The display of these peptides on the cell surface is known as antigen presentation.

ANTIGEN PRESENTING CELLS Pathogens and other exogenous antigens are internalised into the vesicular compartments of antigen presenting cells (APCs), by phagocytosis, endocytosis or macropinocytosis. APCs include professional APCs such as dendritic cells that specialise in stimulating a T cell response; non-professional APCs such as macrophages that can present antigen but have other primary functions and B cells that internalise antigen by receptor-mediated endocytosis of cognate antigen bound to their surface immunoglobulin (sIg).

MHC CLASS I AND CLASS II MOLECULES DELIVER PEPTIDES TO THE CELL SURFACE FROM DIFFERENT CELLULAR COMPARTMENTS WHERE THEY ARE RECOGNISED BY TWO DIFFERENT TYPES OF T CELL

MHC Class I molecules deliver peptides produced in the cytosol to the cell surface where they are recognised by CD8 cytotoxic T cells (TCs). In this way cytotoxic T cells kill cells infected with viruses or bacteria residing in the cytosol.

MHC Class II molecules deliver peptides from the vesicular system to the cell surface where they are recognised by CD4 helper T cells. In this way, helper T cells detect pathogens and their products taken up from the extracellular environment into the vesicular compartment of cells and activate other cells to eliminate the pathogen.

ENDOGENOUS ANTIGEN PATHWAY - MHC CLASS I Generally pathogen-derived peptides that bind MHC Class I molecules are derived from viruses that have infected host cells. Viruses take over the cell's biosynthetic mechanisms to make their own proteins, which are degraded and synthesised as part of normal cell protein turnover.

♦Cytosolic protein degradation takes place in a multicatalytic protease complex called the proteosome (Figure 3.7).

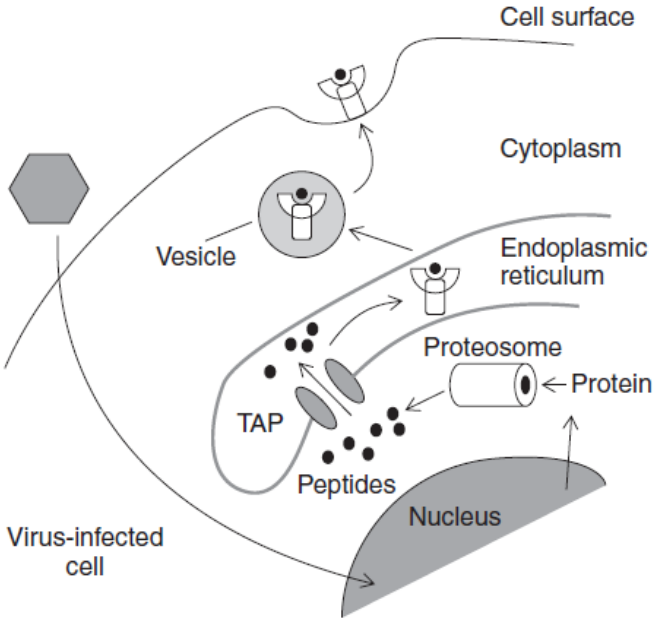

Figure 3.7 Endogenous antigen pathway - MHC Class I.

♦Degraded proteins are transported from the cytosol of a cell into the lumen of the endoplasmic reticulum (ER) via membrane transporter proteins (transporters associated with antigen processing or TAP-1 and TAP-2).

♦In this compartment peptides bind to MHC Class I molecules. Peptide binding is an essential step in the assembly of MHC Class I molecules and this process involves a number of accessory proteins with chaperone-like functions.

♦Peptide:MHC Class I complex is then transported to the cell surface where it can be recognised by cytotoxic T cells.

Some viruses have evolved ways to evade recognition by down-regulating the appearance of peptide:MHC complexes on the cell surface.

EXOGENOUS ANTIGEN PATHWAY - MHC CLASS II Extracellular pathogens and other extracellular antigens that are internalised into endocytic vesicles (endosomes) of APCs are processed in the exogenous antigen pathway (Figure 3.8). The contents of endocytic vesicles are not accessible to cytosolic proteosomes.

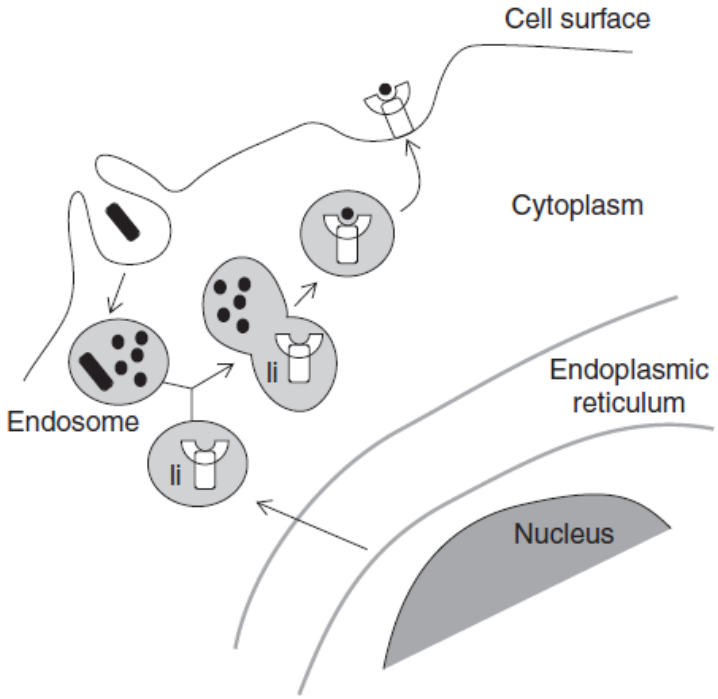

Figure 3.8 Exogenous antigen pathway - MHC Class II.

♦Protein degradation takes place in these vesicles which contain proteases.

♦MHC Class II molecules are prevented from binding peptides in the ER by associating with the invariant chain (Ii), which also targets the molecule to endosomes. MHC Class II molecules reach the endocytic vesicles of macrophages, B cells and dendritic cells.

♦When these endocytic vesicles fuse with peptide containing vesicles, the MHC molecules are loaded with peptides and transported to the cell surface where the peptide:MHC Class II complex can be recognised by helper T cells.

Initiation of an immune response requires activation of helper T cells by APCs with peptide bound to MHC Class II. APCs constantly take up antigen from their environment, and thus bacterial and viral antigens reach the endosomal compartment, even though this is not their normal site of infection. Additionally, several pathogens including mycobacteria and Leishmania replicate in cellular vesicles of macrophages.

RECOGNITION MOLECULES OF THE INNATE IMMUNE RESPONSE The innate immune response recognises microbes by the presence of molecular motifs, which are not present in mammalian cells, using pattern recognition molecules (PRMs). PRMs are constitutively expressed and allow the innate immune response to be constantly effective, as there is no delay required to rearrange microbe specific receptors. Important PRMs are outlined below. A summary of molecular targets of PRMs involved in the innate immune response was presented in Table 1.8.2.

Mannose receptor The mannose receptor binds mannose in microbes and induces phagocytosis. Peptides from the microbe are processed and presented by macrophages, and can activate some T cell subtypes.

Toll receptors Toll-like receptors (TLRs) are a family of closely related proteins that recognise several microbial motifs including peptidoglycan, teichoic acids (found on gram-positive bacteria), LPS (gram-negative bacteria), arabinomannans and glucans. Binding of ligand to TLRs induces expression of immunostimulatory molecules and cytokines important in initiation of the adaptive immune response.

CD14 CD14, found on macrophages, binds LPS, a unique bacterial surface structure found in cell walls of gram-negative bacteria, together with an adaptor protein. When LPS binds CD14, macrophages are activated facilitating destruction of the microbe and secretion of cytokines, triggering a wide variety of immune responses.

Scavenger receptors These receptors are expressed by macrophages and recognise carbohydrates or lipids in bacterial and yeast cell walls.

RECOGNITION MOLECULES OF THE ADAPTIVE IMMUNE RESPONSE

In the adaptive immune response, lymphocytes have unique recognition structures that allow them to see antigen.

♦B cell receptor (BCR)

♦T cell receptor (TCR).

The BCR is surface bound immunoglobulin, and binds conformational epitopes. TCR binds linear epitopes, presented in grooves in MHC molecules. BCR, TCR and MHC proteins are all members of the immunoglobulin superfamily.

B cell receptor complex and B cell co-receptors The BCR complex comprises BCR surface immunoglobulin (sIg) associated with two signaling molecules Iga and IgP (Figure 3.9). Iga and IgP are also required for assembly and expression of sIg. When antigen cross-links sIg, Iga and IgP transduce activation signals into the B cell. Ligation of B cell co-receptors can enhance or inhibit signaling via the Ig-Iga/IgP complex. Optimal activation requires additional signals from helper T cells. Activated B cells differentiate into plasma cells and secrete antibody.

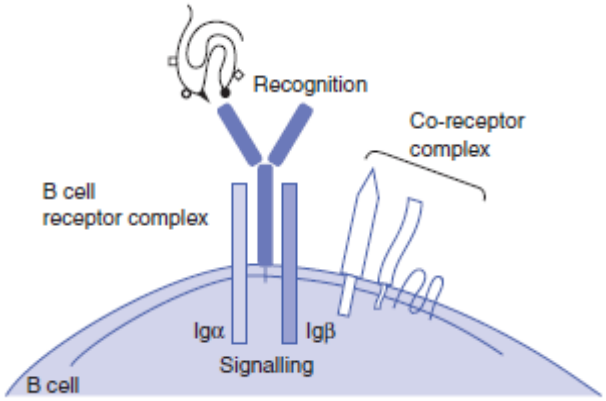

Figure 3.9 The B cell receptor complex and its co-receptor complex. Antibody:antigen interaction.

Antibody-antigen interactions The antigen-binding site of immunoglobulin molecules is composed of variable regions of both heavy and light chains. Antigen specificity of the immunoglobulin is determined by variation in the amino acid sequences of variable regions. Three segments are particularly variable - hypervariable (HV) regions 1-3. Hypervariable segments form surfaces complementary to the antigen: complementarity-determining regions (CDRs) 1-3. Variation in the amino acid sequences of CDRs creates different surface shapes. Antibodies bind epitopes with complementary shapes which fit tightly (Figure 3.9).

T cell receptor (TCR), the TCR complex and co-receptors Most T cells have antigen receptors composed of a and β chains, each with variable and constant regions (V and C regions). A subpopulation of T cells, the function of which is poorly understood, express TCRs consisting of y and δ chains. The TCR complex consists of the TCR associated with CD3 a signaling complex. CD4 or CD8 (co-receptors) play an important role in stabilising the interaction of TCR with MHC-peptide complex.

TCRs interact directly with both the antigenic peptide and some polymorphic regions of the MHC molecule presenting the peptide. CD4 or CD8 help to stabilise the TCR- peptide-MHC interaction which is of very low avidity. CD4 and CD8 distinguish two different functional sets of T cell. CD4 is expressed on helper T cells and binds a nonpolymorphic region of MHC Class II molecules. This effectively restricts helper T cells to recognise peptides appropriately presented on MHC Class II molecules. In contrast, CD8 is expressed on cytotoxic T cells allowing recognition of peptides presented on MHC Class I molecules which is widely expressed.

TCR-peptide-MHC interaction TCRs recognise antigenic peptides bound to MHC molecules on cell surfaces (Figure 3.10(a)). This crucial interaction determines the antigen specificity of adaptive immune responses. Proteins are unfolded, processed into peptide fragments by intracellular proteases, and small peptides are presented in the groove of MHC molecules. Endogenous antigens, and viral proteins, associate with MHC Class I molecules, found on all nucleated cells. Antigens including those derived from pathogens internalised by endocytosis from extracellular fluid, associate with MHC Class II molecules, found on specialised APCs.

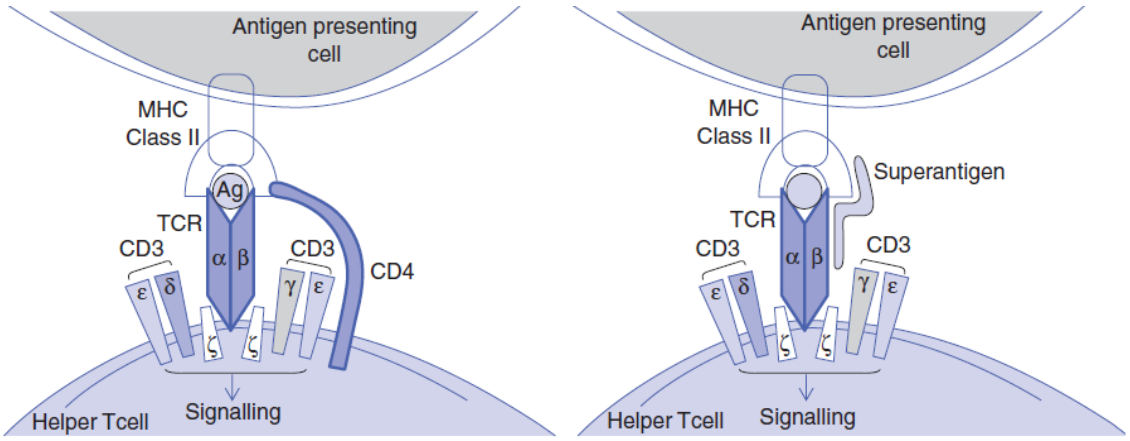

Figure 3.10 (a) T cell receptor complex and antigen:MHC binding. (b) Superantigen binds Yβ region of T cell receptor and MHC Class II molecule.

Superantigen Classical antigens generate peptides that bind the peptide-binding groove of MHC Class II. In contrast superantigens bind directly to MHC without processing. Superantigens bind to relatively non-polymorphic portions of MHC Class II and the Vp region of the TCR (Figure 3.10b). Each superantigen can bind a few families of Vp gene segments, and therefore superantigens can stimulate 2-20% of all T cells. This type of response causes a massive production of CD4 T cell cytokines resulting in systemic toxicity and suppression of adaptive immunity.

Clinically important superantigens include Staphylococcal enterotoxins that cause food poisoning, and the toxic shock syndrome toxin (TSST).

INNATE IMMUNE RESPONSES

The innate immune response detects organisms using pattern recognition molecules (PRMs), which recognise molecules present on microorganisms, but absent from mammalian cells. There is a limited repertoire of PRMs, and they do not vary during the course of the immune response. The innate immune response is capable of mounting a rapid response to an invading microbe and frequently augments the adaptive immune response.

Components of the innate immune response include:

- ***Cells***

— Phagocytes (neutrophils and macrophages)
— Degranulating cells (mast cells and eosinophils)
— NK cells

- ***Proteins***

— Complement
— Natural opsonins (Mannan binding lectin, MBL; C reactive protein, CRP)
— Acute phase reactants

- ***Chemical messengers***

— Cytokines
— Interferons (IFNs)

PHAGOCYTES AND PHAGOCYTOSIS Neutrophils, monocytes and macrophages are all phagocytic cells. Macrophages may develop special phenotypes depending on their location. At sites of inflammation they ay become giant cells and epitheloid cells. Fixed macrophages are found in the sinusoids of the liver and spleen and remove particulate and antibody coated matter from the blood. Specialised macrophages are found in bone (osteoclasts), brain (glial cells) and lungs (alveolar macrophages).

Phagocytosis is the process whereby these cells ingest particulate matter, including pathogens and cellular debris (Figure 3.11). Phagocytic cells recognise debris using PRMs or receptors that bind complement, immunoglobulin or other opsonins. Engagement of receptors on the phagocyte's surface induces formation of pseudopodia, which surround and engulf particles. The ingested particle is contained in a vesicle called a phagosome. Fusion with lysosomes forms phagolysosomes resulting in acidification of the phagosome and release of proteases, and ultimately microbial killing and digestion. Phagocyte activation results in increased oxygen consumption termed the respiratory burst, associated with production of toxic reactive oxygen intermediates (superoxide, hydrogen peroxide).

Phagocytosis can result from engagement of PRMs on phagocytes in the absence of opsonins. However, the process is more efficient in the presence of opsonins including immunoglobulin, which binds to Fc receptors or complement, which engages complement receptors on phagocytes. Natural opsonins do not require activation to acquire activity, behaving as soluble PRMs binding to microbe-specific molecular signatures. These include MBL, which binds mannan residues, a type of carbohydrate not expressed by mammalian cells. CRP is a protein produced in large amounts during episodes of inflammation, and widely measured clinically as a marker of inflammation. It binds to C protein, found on Streptococci, opsonising this group of organisms.

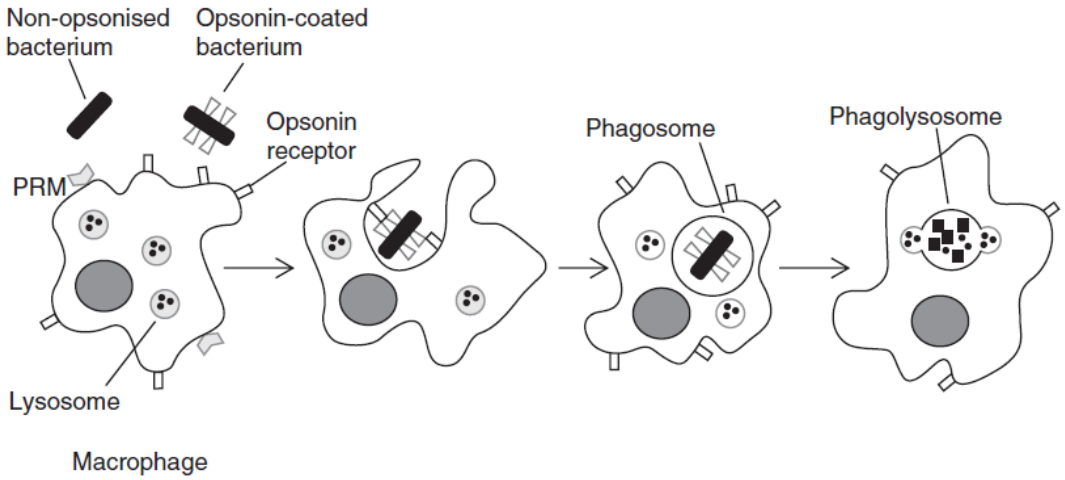

Figure 3.11 Phagocytosis.

INNATE IMMUNITY TO VIRAL INFECTIONS - INTERFERONS Interferons (IFNs) are divided into Type I IFNs (IFN-α. and β) and Type II IFN (IFN-y). Type I IFNs are produced by many cells following viral infection. In contrast, IFN-y is only produced by selected immune cells including some helper T (TH1) cells and NK cells.

Type I IFN production is induced by the double-stranded RNA generated by viruses, but not normally present in mammalian cells. Interferon is produced within hours of infection.

Protective effects of IFN include:

♦Inhibition of viral replication within the infected cell

♦Rendering neighbouring cells more resistant to viral entry

♦NK cell activation

♦Upregulation of viral peptide processing, which bind to MHC Class I molecules. This alerts the adaptive immune response that the cell is infected by a virus.

IFN-y has little direct antiviral activity but has potent immunostimulatory actions. Actions of IFN-y will be described in more detail when T cell function is considered. However, NK cells can also produce IFN-y.

INNATE IMMUNITY TO VIRAL INFECTIONS - NK CELLS NK cells are large granular lymphocytes that constitute about 10% of lymphocytes. They do not rearrange antigen receptor molecules, in contrast to B and T lymphocytes. NK cells can kill without activation by cytokines, but they proliferate in response to IL-2 and become more effective at killing infected cells in the presence of Type I IFNs, IL-12 and IFN-y.

NK cells detect virus-infected cells or tumour cells through a complex system of receptors, some delivering negative and some positive signals. Positive signals stimulate NK cells to kill and they originate from binding abnormal PRMs on infected cells. However, if the cell expresses adequate amounts of MHC Class I, the NK cell receives a negative signal through killer inhibitory receptors (KIRs). This negative signal may override the positive signal to the NK cell - however, an MHC Class I bearing infected cell will later be killed by cytotoxic T cells. Some virally infected cells and tumour cells downregulate MHC Class I, as a mechanism to evade cytotoxic T cells. However, this strategy may result in killing by NK cells.

NK cells also have Fc receptors and bind to antibody-coated target cells. This activates the NK cell, resulting in target cell killing. This is called antibody-dependent cellular cytotoxicity (ADCC).

NK cells kill target cells in two ways that are similar to the mechanisms used by cytotoxic T cells (Figure 3.12). NK cells express Fas ligand (FasL) and thus can bind Fas on target cells inducing programmed cell death or apoptosis in the target cell. Alternatively, NK cells can insert pores into target cells, using perforin. Perforin forms pores by polymerising on the target cell surface. These pores allow

granzymes (proteolytic enzymes) enter the target cell where they degrade host cell proteins. Pores also render the cell susceptible to osmotic lysis. The action of perforin and granzymes can result in apoptosis or necrosis of the target cell.

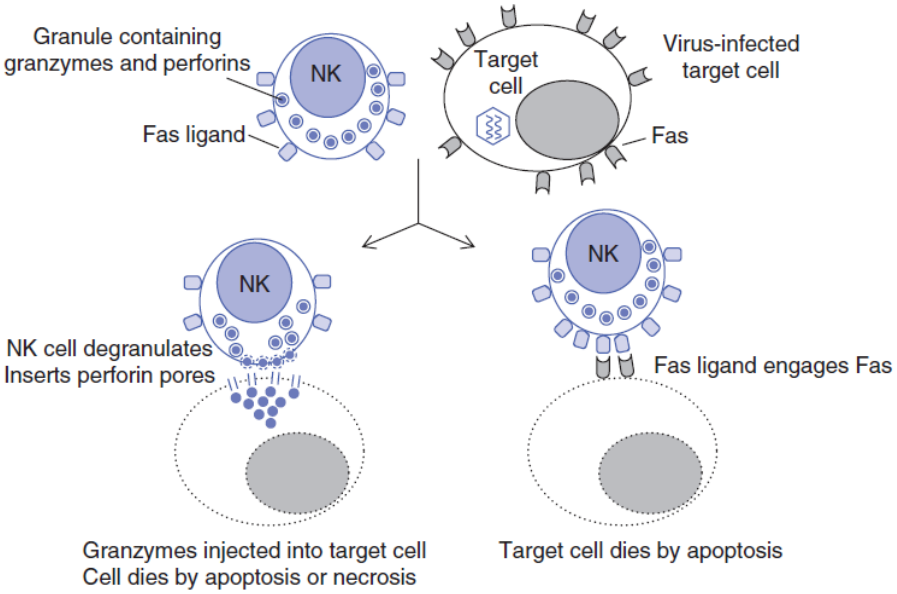

Figure 3.12 NK cells kill target cells by two mechanisms.

In addition to killing target cells, NK cells secrete cytokines including γ-IFN, biasing subsequent adaptive responses towards a cellular immune response, which is the effector mechanism required for recovery from viral infections.

THE COMPLEMENT SYSTEM

The complement system is part of the innate immune system and includes over 20 functionally linked soluble plasma proteins. Complement components are acute phase proteins synthesised in the liver (hepatocytes) and by monocytes. Like other acute phase proteins, synthesis is increased after injury or during inflammation. Activation of complement generates molecules and complexes, important both in defence against bacterial infection and removal of circulating and deposited immune complexes (antibody-antigen). Complement circulates in inactive precursor forms and is activated in a cascade fashion. This is local, occurring on cell membranes or antigen-antibody complexes.

COMPLEMENT ACTIVATION Complement activation can occur via three different pathways:

- ♦Classical pathway
- ♦Lectin pathway
- ♦Alternative pathway.

The classical pathway (including complement components C1, C4, C2) is activated by antibodies bound to antigen (immune complexes). The lectin pathway (MBL, C4, C2) is activated by mannan-containing carbohydrates on bacteria or viruses. The alternative pathway (C3, factor B, factor D properdin) is constitutively minimally active, but is strongly activated when a transiently active complement component binds to polysaccharides on a pathogen. The alternative and mannan binding lectin pathways do not require antibody and therefore represent an early defence against microbial infection. Antibody activation of the classical pathway illustrates how the adaptive immune response harnesses elements of the innate immune response to augment effector responses.

C3 is the central component of the complement system. The first critical step in each pathway involves the generation of an enzyme (‘C3 convertase’) that splits the C3 molecule into C3a and C3b, the biologically active forms of C3. The next step involves the generation of another enzyme (‘C5 convertase’) that splits C5 into C5a and C5b, the biologically active forms of C5. This leads each pathway

into same set of terminal steps generating the cytolytic membrane attack complex (MAC). An overview of these three pathways and their components is found in Figure 3.13.

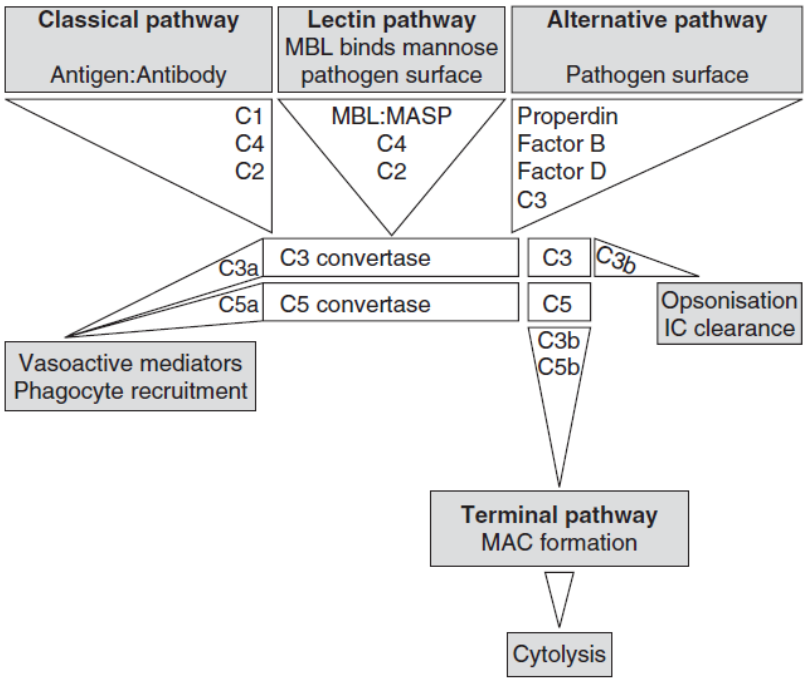

Figure 3.13 Complement activation pathways.

THE BIOLOGICAL FUNCTIONS OF COMPLEMENT

Host defence against infection and foreign antigens Opsonisation C3b coats or opsonises bacteria. Phagocytosis is promoted and enhanced when C3b binds complement receptor 1 (CR1) expressed on neutrophils and macrophages. Chemotaxis and activation of neutrophils C5a attracts neutrophils and monocytes to the site of microbial infection or foreign antigen. C5a also augments cell adhesion, degranulation and activation of the respiratory burst in these cells.

Inflammation and vascular responses The inflammatory peptides C3a and C5a (known as anaphylatoxins) activate mast cells resulting in histamine release. Histamine increases vascular permeability enhancing delivery of cells and proteins of the immune system. Lysis of bacteria and cells The MAC is formed by C5b-C9. This complex forms small pores that puncture cell membranes and cause cell death by lysis.

Solubilisation and phagocytic clearance of immune complexes Immune complexes (ICs) form when the host mounts a vigorous antibody response to an abundant circulating antigen. Immune complexes are potentially harmful if deposited in vessel walls as they lead to inflammatory reactions that damage the surrounding tissue.

Complement affects immune complexes in two ways:

Solubilisation Complement activation on immunoglobulin molecules inhibits immune complex growth by destabilising complex-forming reactions between adjacent immunoglobulin molecules.

Phagocytic clearance Immune complexes bind and activate complement. C3b-coated immune complexes bind to CR1 that is expressed on red blood cells. Phagocytes in the liver and spleen strip and clear RBC-bound immune complexes.

REGULATION OF COMPLEMENT The complement system is a potent mediator of inflammation and is tightly regulated to prevent injury to host cells. Complement cascades are tightly regulated at multiple steps: activated complement components are highly labile with short half-lives. This limits the range of destructive activity close to the activation site. Fluid phase inhibitors including C1-Inhibitor, Factor H, Factor I, C4-binding protein and membrane proteins including CR1, membrane

cofactor protein, decay accelerating factor, and CD59, also play important regulatory roles. Most membrane regulatory proteins are expressed on host cells but not on microbes thus limiting the effects of complement activation to invading microorganisms.

B CELL ACTIVATION

B CELL SELECTION B cells can interact with antigen once surface immunoglobulin is expressed. The type of responses initiated are influenced by

♦B cell maturation stage

♦Signal intensity through the antigen receptor.

The signal intensity also influences whether B cells become re-circulating follicular cells, marginal zone cells or B-1 B cells.

IgM + , IgD— immature B cells are exquisitely sensitive to antigen exposure (Figure3.14). Antigen exposure can result in

♦Clonal deletion - large concentrations of cross-linking antigen promotes cell death.

♦Clonal anergy - cells remain viable but non-functioning following exposure to soluble antigen.

♦Receptor editing - the immunoglobulin receptor alters. Continued RAG gene expression allows further immunoglobulin gene rearrangement. Following receptor editing, B cells which react with self antigen undergo clonal deletion, while those that do not react with self antigen persist. After the IgM+ IgD— stage, RAG expression is switched off and gene rearrangement ceases.

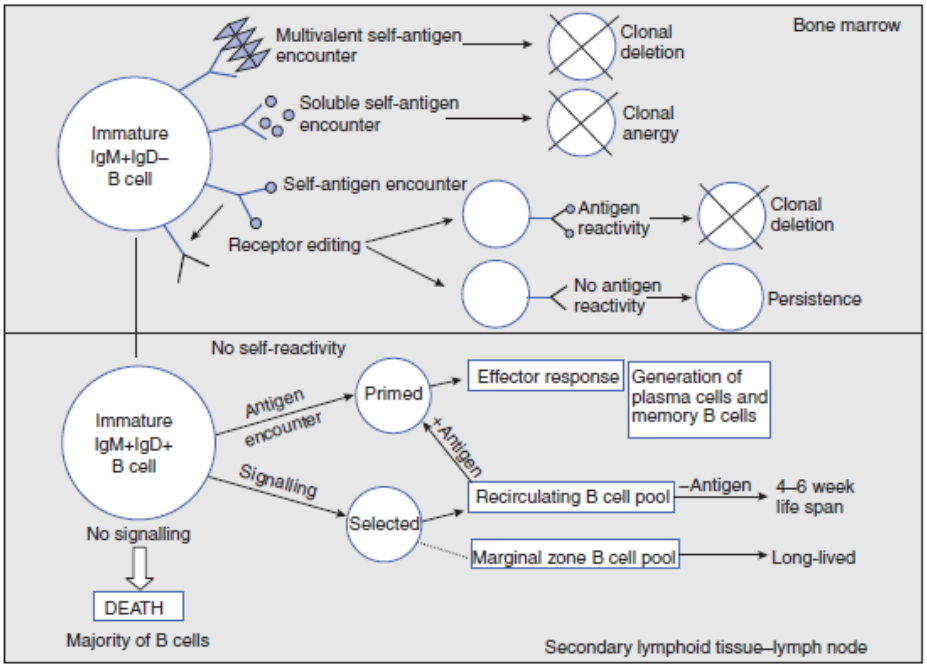

Figure 3.14 B cell selection.

These responses reduce the chances of producing auto-reactive B cells. However, these processes are leaky and self-reactive B cells are usually present in the peripheral lymphocyte pool. Other mechanisms are required to keep these auto-reactive B cells in check. B cells surviving these hurdles are called naive (or 'virgin') newly produced B cells. IgM and IgD are co-expressed on their cell membranes.

B cells are produced so rapidly that the peripheral pool of mature B cells could be fully reconstituted with newly produced B cells every 4-5 days. Only a small minority (<10%) of newly produced B cells survive beyond a few days. Small numbers of newly produced B cells are recruited to the peripheral pool to replace dying mature B cells. Some cells interact with antigen, proliferate and differentiate into antibody-producing plasma cells or memory B cells. Selection of virgin B cells involves signalling, possibly by self-antigens, via the antigen receptor. We do not yet understand how selection signals differ

from activation signals. Variation in signalling strength appears to influence whether B cells become follicular-, marginal zone, or B-1 B cells. Re-circulating B cells can survive up to a few months.

B CELL ACTIVATION The first signal The BCR complex includes the immunoglobulin receptor and associated proteins on B cells (Figure 3.15(a)).

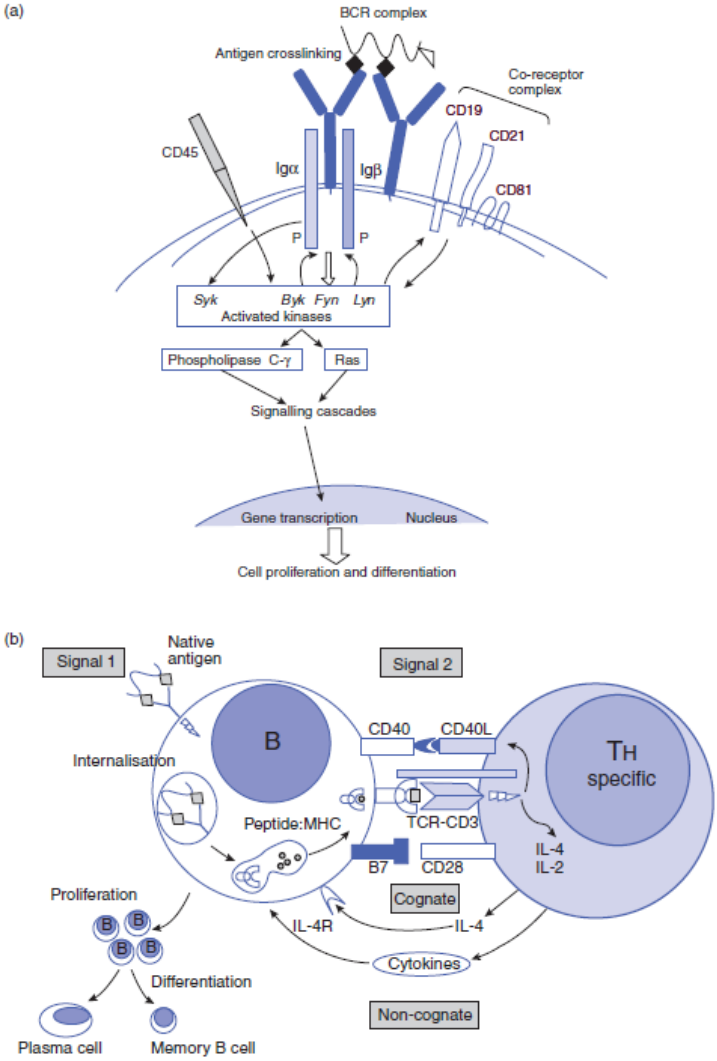

Figure 3.15 (a) Signalling pathways initiated when antigen crosslinks B cell receptor, (b) T/B cell interactions.

♦Iga and IgP (or CD79a and CD79p) maintain immunoglobulin receptor expression on the B cell surface and also transmit signals when antigen binds the immunoglobulin receptor.

♦CD19, CD21 and CD81 (TAPA-1) alter the intensity of signalling generated on antigen binding.

♦ Kinases (Fyn, Blk and Lyn) - enzymes that promote phosphorylation at target sites - associate with the immunoglobulin receptor and generate activation signals.

The signal generated on binding antigen (the primary signal) is usually not adequate to activate B cells to proliferate and differentiate.

The second signal T cell help for B cell responses Secondary signals usually delivered by antigen-specific T cells are typically required for B cell activation. Responses to most protein antigens are T dependent (TD). T cell dependence prevents self-reacting B cells from becoming functional. T cell influences on B cell immune responses include:

♦Promotion of high affinity antibody production

♦Induction of immunoglobulin isotype switching

♦Enhancement of long-term memory responses.

Prior to antigen exposure, only about 1 in 100,000 lymphocytes are specific for a single conventional TD antigen. Secondary lymphoid organs concentrate rare antigen-specific B and T cells, increasing the chance of encounter. Inter-digitating dendritic cells 'trap' antigen-specific T cells re-circulating through the secondary lymphoid tissues and prime them for further antigen encounter. Meanwhile, antigen-specific B cells, on receiving the first antigen-specific signal move to the T cell zones under the control of several chemokines.

Antigen binding to the BCR mediates uptake of bound antigen into the B cell, processing into peptides and packaging with antigen-presenting MHC Class II molecules (Figure 1.14.2b). B cells express MHC Class II from the early stages of maturation.

Presentation of peptide with MHC Class II on the B cell surface brings antigen-specific T cells into close proximity with B cells responding to components of the same antigen. This is called linked recognition.

T cells provide 'help' to B cells through

♦Cognate interaction - primed antigen-specific T cells help B cells to proliferate, and differentiate. The T cell interacting with B cell is specific for the same antigen. Interaction of CD40 expressed by B cells, with CD40 ligand (CD40L) expressed on antigen-specific activated T cells, drives B cell proliferation, antibody class switching and generation of memory responses. Deficiency of CD40L causes immunodeficiency - hyper IgM syndrome - characterised by failure of immunoglobulin class switching. Other B cell/T cell interactions are important in generating TD B cell responses, including B7/CD28.

♦Non-cognate - reactions are not antigen-specific. Once activated through cognate interactions, B cells can respond to cytokines secreted by activated T cells. T cell cytokines affect B cell proliferation (IL-4), immunoglobulin class switching (IL-4, transforming growth factor-β (TGF-β) and IFN-y), and differentiation into plasma cells (IL-6).

B cell proliferation hugely increases the frequency of antigen-specific cells over a few days. B cells do not proliferate indefinitely but rather differentiate, in a manner determined by the type of antigen, the type of T cell cognate interactions and the cytokine environment.

Activated B cells can differentiate into the following:

♦Short-lived plasma cells - produce IgM predominantly.

♦Follicular B cells - relocate from the T zone of secondary lymphoid tissues into the follicles where they establish a germinal centre reaction. Somatic hypermutation occurs in rapidly dividing germinal centre B cells (centroblasts). Variable regions of immunoglobulin genes are altered. Some B cells improve the affinity with which they bind antigen. This is a short-lived event in the immune response and cells that have undergone somatic hypermutation (centrocytes) subsequently require signals from antigen and T cells to survive. Only B cells that receive both these signals - that is, responsive to native antigen and not self-reactive - survive.

Some surviving centrocytes differentiate to become:

♦Long-lived plasma cells - relocate to bone marrow and the lamina propria of the gut, or

♦Memory B cells - colonise the marginal zones of secondary lymphoid tissues, responding with faster and bigger responses on second and subsequent exposures to antigen.

T INDEPENDENT B CELL ACTIVATION The polysaccharide capsules of many pyogenic bacteria have distinct repeating structures. Antigen receptor binding of highly repetitive epitopes induces extensive cross-linking of the BCR complex and the B cell may be activated without additional signals. Activated complement and macrophage factors can also promote B cell activation. Antigens associated with lipopolysaccharide (e.g. gram-negative bacteria) are potent activators of complement and macrophages. If these factors can provide an adequate second signal to B cells during antigen-specific activation the requirement for T cells in B cell activation may be bypassed. Both LPS- and

polysaccharide-based antigens are classed as T independent (TI) because T cells are not essential for B cells to make antibody responses.

TI-type responses are either

♦TI-1 - responses generated by LPS and similar antigens or

♦TI-2 - responses generated by polysaccharide antigens. Marginal zone B cells may preferentially respond to TI-2 antigens.

Different mechanisms allow both types of antigen to be TI. Although not essential, T cells can regulate B cell responses induced by TI antigens.

HELPER T CELL ACTIVATION Mature, but antigen-naive T cells recirculate between blood and lymphoid tissue until they encounter their specific antigen. On encountering their cognate peptide:MHC complex on the surface of an activated professional antigen presenting cell (APC) in lymphoid tissues, naive T cells become activated, proliferate and differentiate. T cells are subdivided into cytotoxic T cells (Tcs) which are usually CD8+ and helper T cells (THs) which are usually CD4 + . Helper T cells are further divided based on their profile of cytokine production.

Initial encounter with antigen initiates a primary immune response. Once activated, primed or memory T cells respond quickly and effectively to subsequent antigen exposure.

ACTIVATION OF NAIVE T CELLS REQUIRES TWO SIGNALS

Signal 1 Engagement of T cell receptor

Signal 2 Engagement of co-stimulatory molecules

Naive T cells recognise peptide:MHC complex on the surface of APCs, however, ligation of TCR and CD4 or CD8 is insufficient to activate naive T cells. A second co-stimulatory signal is required. Dendritic cells are professional APCs that express MHC and costimulatory molecules required to activate naive T cells. Non-professional APCs (B cells and macrophages) are capable of activating T cells previously primed by dendritic cells, including memory T cells. The interaction between peptide:MHC and TCR confers antigen specificity on the immune response. However, this interaction is of low avidity, and must be stabilised by pairs of adhesion molecules (such as LFA-1 and ICAM-1) and co-stimulatory molecules (Figure 3.16).

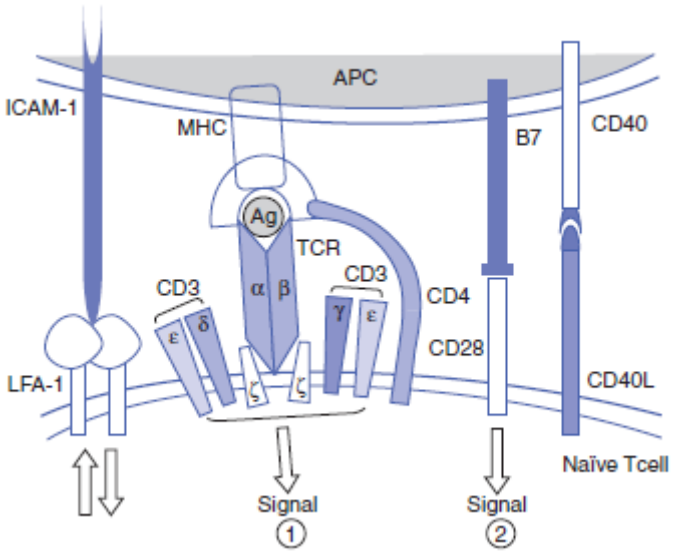

Figure 3.16 Interaction of naive T cell with an APC.

In lymphoid tissues, T cells bind transiently to APCs, sampling large numbers of peptide:MHC complexes. If the naive T cell does not encounter specific antigen it dissociates from the APC and continues to recirculate. On recognising its peptide: MHC, signalling through the TCR increases the binding affinity of adhesion molecules, stabilising the antigen-specific T cell/APC interaction. In the

presence of additional co-stimulation, the T cell is activated, proliferates and differentiates, producing armed effector T cells.

Co-stimulatory molecules

Activation of naive T cells requires a co-stimulatory signal, usually delivered by the same APC that delivered the antigen-specific signal. Co-stimulatory molecules are upregulated on activated dendritic cells following ingestion of antigen and migration to lymphoid organs. APCs engage in a co-stimulatory dialogue with T cells involving several different pairs of molecules. This is initiated through binding of B7 molecules on activated APCs to CD28 on T cells. CD40 ligand expressed on the activated T cell binds CD40 on APCs providing further potent co-stimulation.

Antigen binding by TCR in the absence of co-stimulation induces anergy (T cell remains viable, but refractory to activation by antigen), or programmed cell death (apoptosis). As co-stimulatory molecules are not widely expressed, this helps to maintain self-tolerance in peripheral tissues.

T CELL ACTIVATION AND INTRACELLULAR SIGNALLING PATHWAYS Activation of T cells by antigen together with co-stimulation results in sequential activation of enzymes and subsequent signalling cascades. These activation signals finally initiate transcription of genes and production of proteins that direct T cell proliferation and development of effector functions, including genes encoding IL-2 and the α-chain of the IL-2 receptor (Figure 3.17). When IL-2 binds the IL-2 receptor T cells proliferate, and then differentiate into armed effector T cells. Availability of potent immunosuppressives that inhibit IL-2 production has greatly enhanced the success of clinical transplantation.

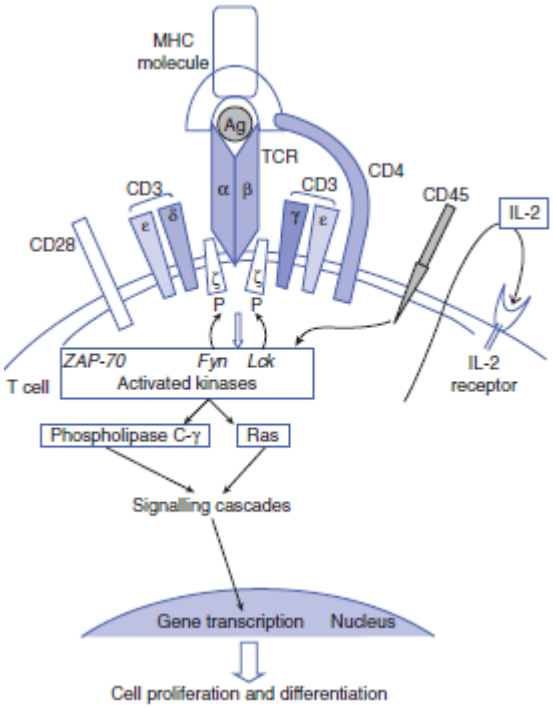

Figure 3.17 Intracellular signalling pathways of a T cell

PROLIFERATING T CELLS DIFFERENTIATE INTO ARMED EFFECTOR CELLS THAT DO NOT REQUIRE CO-STIMULATION TO ACT Once activated T cells differentiate into armed effector cells synthesising molecules required for specialised functions. CD8+ T cells become fully functional cytotoxic T cells (TCs) while CD4+ cells become fully effective helper T cells (THs). Once differentiated, encounter with specific antigen results in immune attack without the need for further co-stimulation. Clearly cytotoxic T cells must be able to kill any virus-infected cell regardless of expression of co-stimulation molecules. CD4 T cells must be able to activate B cells and macrophages that have taken up antigen.

Interactions between effector T cells and target cells are initiated by transient interactions through adhesion molecules (LFA-1 and CD2). If TCR binds antigen the binding affinity increases, allowing enough time for effector T cells to release effector molecules locally on the target cell. Soluble cytokines and membrane-associated molecules act in combination to mediate T cell effector functions.

ACTIVATION AND DIFFERENTIATION OF NAIVE CD4 T CELLS INTO TH1 OR TH2 CELLS DETERMINES THE CHARACTERISTICS OF THE SUBSEQUENT ADAPTIVE RESPONSE Once activated, naive CD4 T cells differentiate into helper T cells, which can be further differentiated based on the profile of cytokine production. Helper T cell cytokine production

orchestrates the subsequent immune response. The functional fate of the helper CD4 T cell is decided during clonal expansion, however, the factors determining the type of helper T cell produced are unclear. Initial work classifying helper T cells, performed using cloned murine T cell lines demonstrated a clear division into two subtypes, TH1 or TH2 cells, with distinct cytokine production profiles. Human studies suggest that TH1 and TH2 cells are extreme ends of a spectrum, with many T cells having intermediate cytokine profiles, however, the paradigm remains useful. TH1 cells are essential for cell mediated immunity and also support production of some opsonising antibody isotypes. TH2 cells are essential for production of IgE and also support production of other antibody isotypes. TH1 cells suppress the development of TH2 cells and vice versa. T cell help is provided by

- ♦Molecular interactions that occur during cell-cell contact
- ♦Production of cytokines.

TH1 cells

- ♦Activate macrophages that are infected by or have ingested pathogens.
- ♦ Secrete IFN-7 which activates macrophages, and lymphotoxin (LT-a or TNF-β) which activates macrophages and inhibits B cells.
- ♦Express CD40L, which interacts with CD40 on macrophages sensitising the macrophage to IFN-7.

TH2 cells

TH2 cells are required for IgE production and also contribute to production of other antibody isotypes. T cell help is required for B cells to produce antibody in response to T-dependent antigens, for isotype switching and affinity maturation of the antibody response. TH2 cells

- ♦Produce cytokines involved in B cell proliferation and maturation - including IL-4, IL-5 and IL-6
- ♦Express CD40L, which binds CD40 on B cells inducing B cell proliferation
- ♦Secrete IL-10 which inhibits macrophages.

Both TH1 and TH2 cells produce IL-3 and GM-CSF which stimulate production of macrophages and granulocytes - important non-specific effector cells in both humoral and cell mediated immunity.

ACTIVATION OF MACROPHAGES BY ARMED CD4 TH1 CELLS

When macrophages ingest organisms, they form vesicles around the organisms. In the absence of help from TH1 cells, macrophages are relatively inefficient at killing these organisms. Resistant pathogens (e.g. Mycobacteria) can only be killed by activated macrophages. Macrophage activation requires both IFN-7 produced by the T cell and interaction of macrophage CD40 with T cell CD40L (Figure 3.18). This process is CENTRAL to the host response to pathogens that proliferate in macrophage vesicles.

Activated macrophages upregulate expression of MHC Class II molecules, B7 molecules, CD40 and TNF receptors, thus increasing their effectiveness as APCs. TNF- a acts with IFN-7 in the induction of toxic oxygen radicals. Activated macrophages also secrete IL-12, which enhances differentiation of naive CD4 T cells into TH1 effector cells.

Activated TH1 cells produce cytokines that activate the macrophage and coordinate the immune response to intravesicular pathogens. TH1 cells enhance recruitment of phagocytic cells to the site of infection. IL-3 and GM-CSF stimulate production of phagocytic cells in the bone marrow. TNF-α. and

TNF-β promotes adhesion of phagocytes to local vascular endothelium. Macrophage chemotactic factor-1 (MCP-1) attracts phagocytic cells to the site of infection.

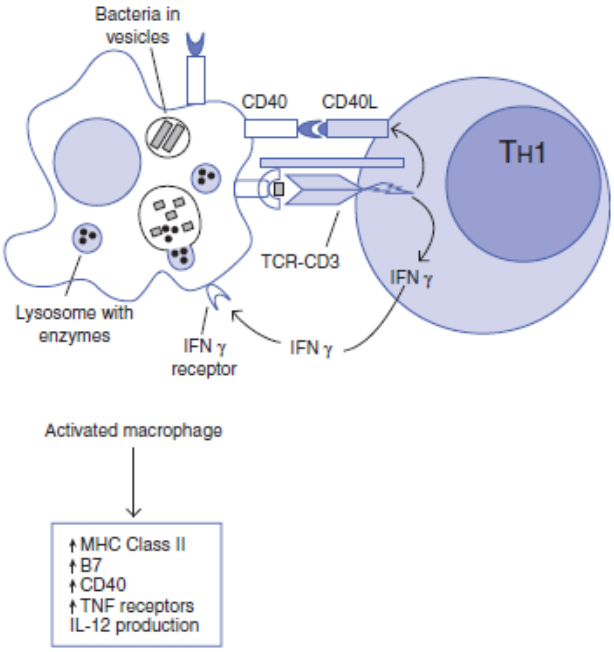

Figure 3.18 TH1 CD4 cells activate macrophages to kill bacteria living in its vesicles.

Chronically infected macrophages lose the ability to kill intracellular bacteria. Fas ligand (FasL) expression or TNF-β produced by TH1 cells can kill these macrophages, releasing engulfed bacteria to be taken up and killed by other macrophages.

When antigen persists, for example, due to microbial resistance a chronic TH1 dominated cellular response ensues. This characteristically produces a central area of infected macrophages surrounded by activated lymphocytes, called a GRANULOMA.

Regulation of macrophage activation avoids damaging healthy tissue

Tight regulation of macrophage activity by TH1 cells allows specific and effective deployment of potent mechanisms of activated macrophages, while minimising local tissue damage. Control mechanisms include the following:

♦T cell synthesis of IFN-y limited by short half-life of mRNA encoding IFN-y.

♦Focal delivery of IFN-y to point of contact of TH1 cell with macrophage limits effect to infected macrophage.

♦Inhibition by cytokines including TGF-β, IL-4, IL-10, IL-13 which are produced by TH2 cells.

EFFECTOR T CELLS FALL INTO THREE FUNCTIONAL CATEGORIES

Protective immunity against pathogens in macrophage intracellular vesicles requires activated CD4 T cells. TH1 cells activate macrophages enabling them to kill these organisms.

Extracellular pathogens include bacteria and multicellular parasites. Sterilising immunity to bacteria relies on the humoral immune response, and T cell help is required for optimal antibody production. IgE production, requires help from TH2 cells, which provides some protection against multicellular parasites.

Intracellular pathogens multiplying in the cytoplasm activate cytotoxic T cells (TCs). Cytotoxic T cells kill infected target cells in minutes by releasing preformed proteins from their vesicles or inducing apoptosis (Figure 3.19).

ACTIVATION OF NAIVE CD8 T CELLS Naive CD8 T cells differentiate into cytotoxic T cells when they first encounter antigen (peptide: MHC) on the surface of antigen presenting cells (APCs) in lymphoid tissues (Figure 3.20). Proliferation and differentiation of cytotoxic T cells into armed effector cells depends on adequate co-stimulation and production of IL-2, in addition to TCR- peptide:MHC binding.

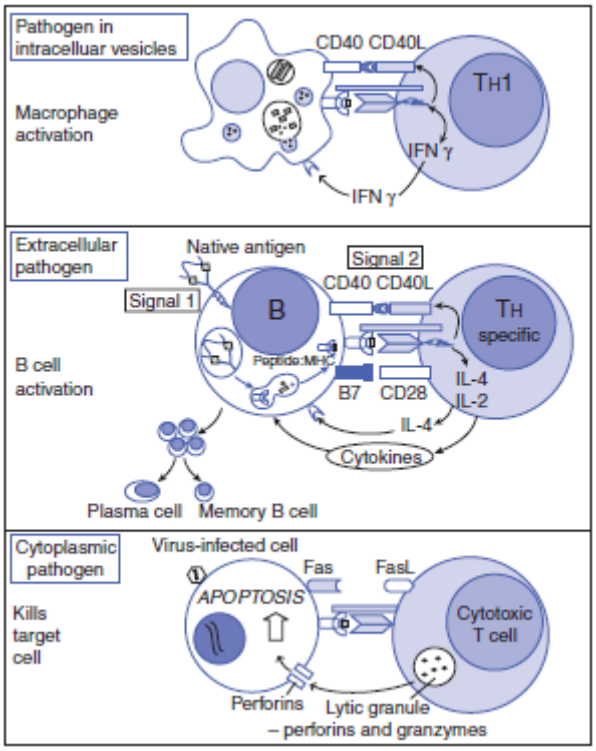

Figure 3.19 Three main classes of effector T cell.

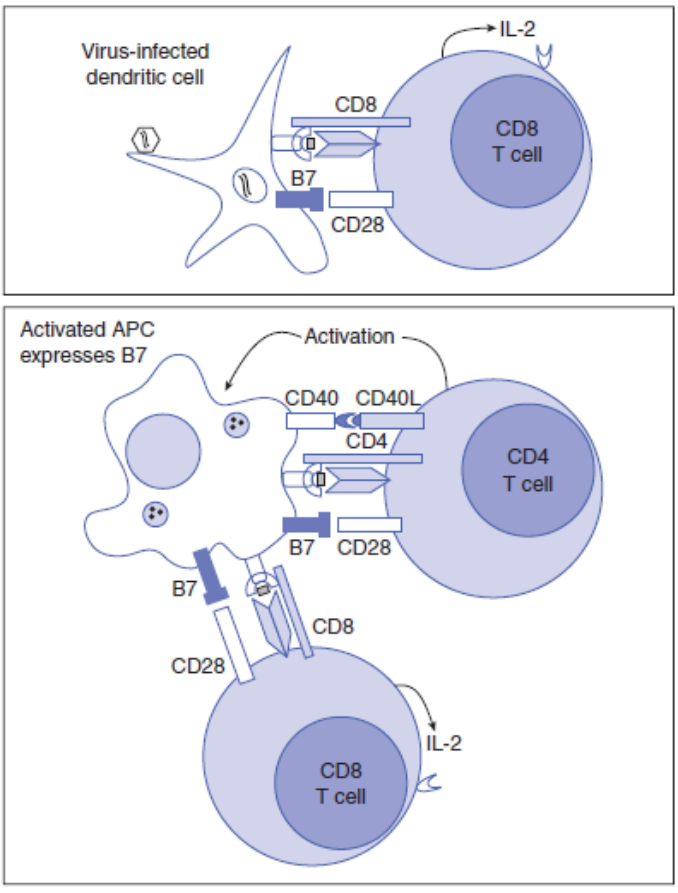

Figure 3.20 Activation of naive CD8 T cells.

Dendritic cells, macrophages and B cells express both classes of MHC as well as the co-stimulatory cell-surface molecules required. The double requirement of co-stimulation and IL-2 production can be met in two ways:

♦Dendritic cells, which have high co-stimulatory activity, can directly stimulate CD8 + T cells to synthesise IL-2, which drives their own proliferation and differentiation.

♦During priming, helper T cells and naive CD8 T cells recognise related antigens on the surface of the same APC. The helper T cell induces higher expression of co-stimulatory molecules on the APC, which in turn activates the CD8 T cell to make IL-2.

ACTIVATION OF ARMED EFFECTOR CYTOTOXIC T CELLS Once a CD8 T cell has differentiated into an armed effector cytotoxic T cell, response to its specific antigen does not require co-stimulation. This makes sense, as cytotoxic T cells mustbe able to act on any cell infected with a virus whether or not it can express co-stimulatory molecules.

Peptides derived from intracellular (cytoplasmic) microbes, are processed and presented bound to MHC Class I molecules on the cell surface, marking the cell for killing by cytotoxic T cells. Interactions between adhesion molecules on cytotoxic T cells and on the target cell allow the cytotoxic T cell to scan cell surfaces for the presence of specific peptide:MHC complexes. The TCR binds the peptide:MHC Class I complex. CD8 binds the nonpolymorphic region of MHC Class I molecule.

CYTOTOXIC MECHANISMS OF ARMED EFFECTOR CYTOTOXIC T CELLS Upon activation, cytotoxic T cells induce apoptosis or necrosis of a virus infected cell by two mechanisms (Figure 3.21).

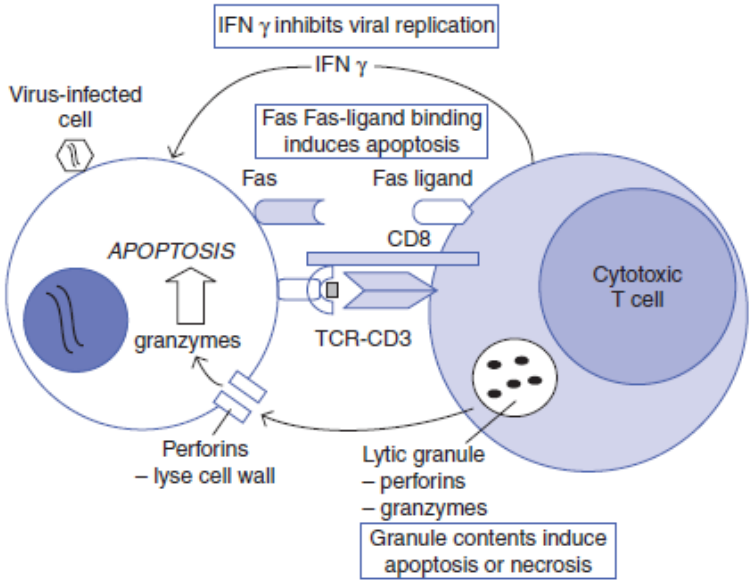

Figure 3.21 Mechanisms of cytotoxicity in cytotoxic T cells.

Calcium dependent release of lytic granules The granules of TCs contain effector proteins: perforin and proteases called granzymes. When TCs recognise antigen on a target cell, the lytic granules release effector molecules by a calcium-dependent mechanism. Perforins polymerise to form a pore through which the granzymes enter the target cell and can activate a cascade of enzymes inducing apoptosis. This mechanism may also result in cell death by necrosis. TCs can kill their targets rapidly because they store preformed cytotoxic proteins that reside in an inactive form in the lytic granules.

Fas ligand also induces apoptosis Cytotoxic T cells (TCs) can also kill target cells in a perforin-independent manner. Infected nucleated cells upregulate Fas expression. Activated TCs upregulate their expression of FasL. Ligation of Fas on target cells by FasL activates caspases that induce apoptosis in the target cell.

Activated cytotoxic T cells release cytokines In addition to releasing perforins and enzymes, cytotoxic T cells also produce immuno- stimulatory cytokines IFN-y, TNF-a and TNF-β.

♦IFN-y upregulates the expression of MHC Class I molecules and slightly inhibits viral replication. IFN-y also activates macrophages and recruits them to the site of infection as effector cells and as APCs.

♦TNF -a and TNF-β synergise with IFN-y in macrophage activation.

THE ACUTE PHASE RESPONSE (APR)

Dramatic changes in vasculature, metabolism, temperature and plasma protein composition occur in response to tissue damage. Antimicrobial activity is enhanced. Pro-inflammatory cytokines (IL-1, IL-6 and TNF-α) released by phagocytes are the dominant mediators of these effects. Effects occur within hours and contribute to protection before and during the adaptive immune response. Adverse effects occur if the APR is inappropriately exaggerated or prolonged (Figure 3.22).

Key features include:

♦Fever - inflammatory cytokines re-set hypothalamic temperature control. Microbial growth is impaired and specific immunity is more efficient at higher temperatures.

♦Increased metabolism - release of energy from muscle and fat stores increases temperature. Weight loss is common.

♦Vascular changes - TNF-α increases vascular permeability, facilitating movement of inflammatory cells and molecules into damaged tissue. Procoagulant effects localise inflammation at sites of injury.

During the APR the concentrations of many plasma proteins are altered - known as acute phase reactants. Some have anti-microbial properties.

Pro-inflammatory cytokines induce increased liver cell synthesis of the following proteins:

♦CRP - natural opsonin

♦MBL - natural opsonin

♦Complement proteins (C3, C4)

♦Ferritin - reduces levels of free iron which impedes some microbial growth

♦Fibrinogen - procoagulant effects.

Immunoglobulin levels also increase slowly during chronic inflammation.

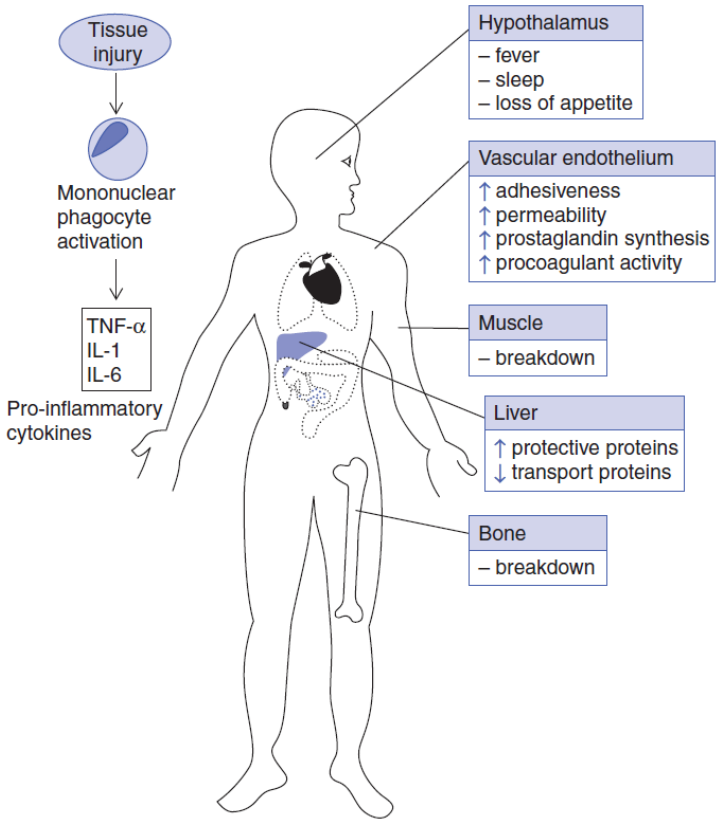

Figure 3.22 The acute phase response.

Reduced production of other proteins occurs in the APR:

♦Albumin - hypoalbuminaemia can be pronounced in inflammatory states.

♦Transferrin - like ferritin, reduces iron required for microbial growth.

The net effect is to shift hepatic protein synthesis towards production of protective proteins and away from storage and transport proteins. Elevation of plasma proteins, especially fibrinogen and immunoglobulins, increases the Erythrocyte Sedimentation Rate (ESR) in inflammatory states. Measurement of CRP is commonly used to monitor inflammation. Distinct patterns of plasma protein variations occur in different conditions.

CYTOKINES

Cytokines are small soluble proteins produced by cells (mainly of immune origin) that alter behaviour of that cell itself or of other cells.

Cytokines are categorised according to the cell of origin into:

♦Interleukins (IL) - produced by blood cells and primarily influence leucocytes

♦Lymphokines - produced by activated lymphocytes

♦Monokines - produced by activated mononuclear phagocytes.

Common structural features allow cytokines to be classified into distinct families:

♦Interferons - IFN-α and β, IFN-γ

♦Chemokines - IL-8, RANTES etc.

♦TNF family - TNF-α and β

♦Haemopoietins - IL-2, IL-4, IL-6, granulocyte-colony stimulating factor (G-CSF), granulocyte monocyte-colony stimulating factor (GM-CSF), IL-3, IL-7.

Cytokines exert their effects through interaction with cytokine receptors on cell surfaces, and often use common signaling pathways within cells. Families of structurally related receptors interact with families of cytokines (above).

Cytokines can also be categorised according to the major effector functions they mediate.

♦Innate immune response cytokines - initiate early immune responses. They also influence the type of subsequent specific immune response.

♦Regulation of lymphocyte function - cytokines induce, direct and regulate lymphocytic responses.

♦Inflammatory cell activation - mediate effector functions of macrophages and lymphocytes.

♦Regulation of cell movement - chemokines are a distinct group of cytokines that share structural and functional characteristics. Chemokines are secreted by many cell types and influence the movement of immune cells.

♦Haemopoietic growth factors - a number of cytokines influence haemopoietic differentiation.

Cytokines have a multitude of effects, but a number of features are common:

♦Short duration of action - half-life is short and degradation is rapid.

♦Locally active - most work on the cell of origin or on cells in the local microenvironment only. Others, like the haemopoietic factors and TNF-α act on distant cells.

♦Multiple effects - most cytokines have many rather than one action (e.g. TNF-α - neutrophil activation, vascular effects, hepatic effects). Cytokine concentration also influences the pattern of the response.

♦Overlapping effects - the same effect can be mediated by a number of cytokines (e.g. pro-inflammatory effects of IL-1 and TNF-α). This is an obstacle to the development of cytokine-directed therapies universally effective in inflammatory diseases.

All these factors make measurement of circulating cytokines both difficult and of dubious relevance in most situations.

Table 3.6 is not a complete list of all known cytokines but, rather highlights well- documented effects of some cytokines with critical roles in the induction, direction and regulation of inflammatory and immune responses.

Table 3.6 Cytokines and their functions

CYTOKINE	SOURCE	ACTIONS
Innate immunityand inflammatory responses		
IL-1	Macrophage	Vascular endothelial activation; neutrophil mobilisation, activation; APR activation; non-specific lymphocyte activation; increases IL-6 production
IL-6	Macrophages,	APR induction; lymphocyte activation; enhanced
	T cells	antibody production
TNF-a	Macrophages,	Vascular endothelial activation; neutrophil activation;
	NK cells	APR induction; energy release
IFN-a and β	White cells (a),	NK activation; enhanced antigen recognition; inhibition
	Fibroblasts (β)	of viral replication
IFN-7	T cells, NK cells	Macrophage, endothelial cell and NK activation
IL-5	TH2 T cells	Eosinophil growth and survival; B cell differentiation to IgE production
IL-8	Macrophages	Neutrophil chemoattractant
MIP-1 analogues	Macrophages, T cells	T cell and monocyte chemoattractant
MCP	Macrophages	Monocyte chemoattractant
RANTES	T cells	Memory T cell chemoattractant
Eotaxin	T cells	Eosinophil chemoattractant
Lymphocyte regulation		
IL-2	T cells	Lymphocyte proliferation and differentiation; IL-15 similar effects
IFN-7	T cells, NK cells	Enhances CTL and TH1 pattern responses
IL-4	T cells, mast cells	TH2 cytokine; B cell activation and class switching to IgE; inhibit TH1 responses; IL-13 similar effects
IL-10	T regulatory cells	Inhibit TH1 responses; suppresses cytokine production by macrophages
TGF-β	T regulatory cells	Anti-inflammatory; pro-fibrotic
IL-12	Macrophages	NK cell activation; promote TH1 responses
Haemopoietic growth factors		
IL-3	Thymic epithelium, T cells	Early haemopoiesis
IL-7	Bone marrow stromal cells	Lymphocyte development
G-CSF	Fibroblasts	Neutrophil development
GM-CSF	Macrophages, T cells	Myeloid-monocytic development

INFLAMMATION

Inflammation is the reaction of living tissue to injury or infection, and may be acute or chronic. Acute inflammation may be followed by resolution, or if the stimulus persists may become chronic (Figure 1.7.1). Clinically, acute inflammation is characterised by heat, redness, swelling and pain and often loss of function.

Inflammation has many beneficial functions including:

- ♦Dilutes and removes toxins
- ♦Limits spread of bacteria

♦Facilitates influx of neutrophils, complement, opsonins and antibodies
♦Provides a supply of inflammatory mediators
♦Ensures an increased supply of nutrients for cells
♦Promotes initiation of the immune response
♦Initiates the healing process.

However acute inflammation also has harmful effects. Swelling may have a mechanical effect - for example, in acute epiglottitis where the airway may become obstructed by the swollen epiglottis. Swelling can impair function directly or by impairing blood flow, particularly when tissue expansion is limited (e.g. in intracranial inflammation). Inflammation also contributes to tissue damage. When acute inflammation fails to resolve, chronic inflammation can cause considerable tissue damage, which may result in scarring and loss of tissue function.

INITIATION OF INFLAMMATION. Inflammation results from a variety of insults, which lead to production of pro-inflammatory mediators. The nature of these mediators affects the nature of the inflammatory response. The type and severity of the injury and the genetic makeup of the individual affect inflammation.

Inflammation may result from:
♦Physical injury - trauma, burns
♦Infection - organisms and toxins
♦Allergy
♦Autoimmune disease.

Components of the innate and adaptive immune response play pivotal roles in the inflammatory process.

THE INFLAMMATORY RESPONSE. Injury damages cells resulting in release of proinflammatory mediators such as prostaglandins and leukotrienes and leakage of cell contents. Bacterial components and toxins provide additional proinflammatory stimuli. Mast cell degranulation results from physical trauma and complement activation, releasing histamine. Tissue macrophages ingest particles and debris, become activated and release a number of proinflammatory cytokines such as IL-1, IL-6 and TNF-α. Macrophages also produce chemokines, which attract specific leucocytes to the site of injury.

Prostaglandins, histamine and complement components (particularly the anaphylatox- ins C3a and C5a) cause vasodilation and increased vascular permeability. This increases extravasation of fluid, including macromolecules, which do not cross a normal vascular bed. Monokines also alter vascular endothelium locally, making it more ‘sticky’ by upregulating adhesion molecules, and increasing expression of procoagulant molecules. Enhanced clotting in local vessels helps to stop bleeding and also limits the ability of bacteria to spread.

Leucocytes migrate to the area of inflammation, with neutrophils arriving rapidly, followed by monocytes and lymphocytes hours to days afterwards. Leucocytes normally travel in the centre of vessels, due to laminar flow. Leucocytes make contact with endothelium intermittently, rolling for some distance, and then detaching if no further adherent stimuli are encountered. If the rolling leucocyte encounters inflamed endothelium expressing activated integrin adhesion molecules, integrin-mediated firm adhesion ensues (tight binding). The leucocyte can then migrate through the endothelium, via the interaction of additional adhesion molecule pairs (diapedesis). Leucocytes reach the site of tissue injury by chemotaxis (directional cell migration) along gradients of chemotactic mediators. Chemotactic mediators which attract neutrophils include anaphylatoxins, leukotrienes, chemokines such as IL-8 (produced by macrophages) and bacterial products, particularly formyl peptides (Figure 3.23).

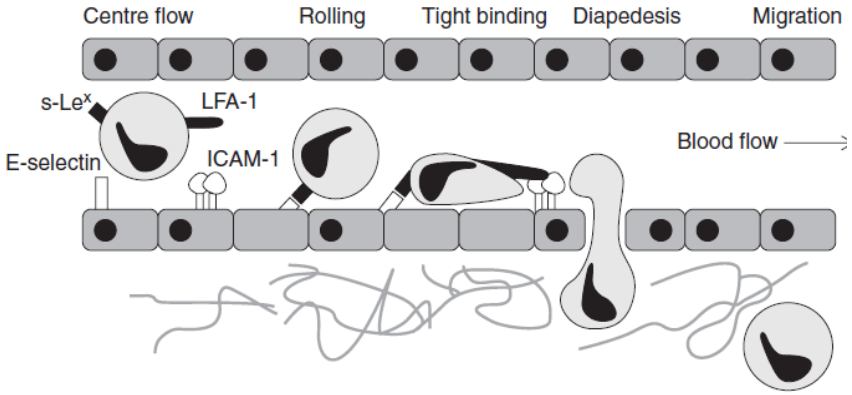

Figure 3.23 Multi-stage leucocyte–endothelial interaction.

The precise composition of the inflammatory infiltrate is influenced by the initiating stimulus. Suppurative inflammation is commonly seen in response to infection with pyogenic bacteria. The inflammatory infiltrate is neutrophil dominated, and pus formation may result. Pus is a semi-liquid mixture containing bacteria, damaged cells and neutrophils. It is green because of the high content of neutrophil myeloperoxidase. A localised collection of pus is called an abscess. However, mast cell degranulation plays a key role in initiating this type of inflammation, and the early phase is dominated by tissue oedema with a later influx of eosinophils. Pus formation does not occur.

Later in the course of inflammation monocytes and lymphocytes enter, attracted by chemokines released from macrophages and other cells in the inflammatory infiltrate.

If the stimulus is removed or eradicated acute inflammation may resolve, often leaving no tissue injury. Once the stimulus is removed, the rate of arrival of new cells decreases rapidly. Leucocytes present at the inflammatory site die by apoptosis and are removed by resident macrophages. Fibroblasts repair the connective tissue, and breaches in epithelium heal.

The physiological and cellular events underlying inflammation give rise to the clinical features outlined in Table 3.7.

Table 3.7. Clinical and physiological features of acute inflammation.

CLINICAL EFFECT	PHYSIOLOGICAL EFFECT	MEDIATORS
Heat	Increased blood flow Vasodilation	Histamine Prostaglandins (some)
Redness	Vasodilation	Anaphylotoxins (C3a, C5a)
Swelling	Vasodilation Extravasation of fluid	Histamine Bradykinin Anaphylatoxins Leukotrienes
Pain	Nerve stimulation Pressure on nerve endings	Prostaglandin E2 Bradykinin

Acute inflammation enhances antigen presentation to the immune system, eliciting an immune response to infecting organisms. APCs including dendritic cells and macrophages at the site of inflammation take up antigen and become activated. These APCs then migrate to regional lymph nodes where the chances of encountering antigen specific T and B cells are substantially increased. In the local nodes lymphocyte proliferation and differentiation occurs. Activated T cells then migrate back to the tissues, while B cells mature into plasma cells and produce antibody in the lymph nodes or bone marrow.

CHRONIC INFLAMMATION. Chronic inflammation usually follows unresolved acute inflammation, usually because of persistence of infection, allergen or other stimulus. Occasionally chronic inflammation can occur without preceding acute inflammation in some types of autoimmune disease. Chronic inflammation is characterised by ongoing tissue damage occurring at the same time as healing and repair (Figure 3.24).

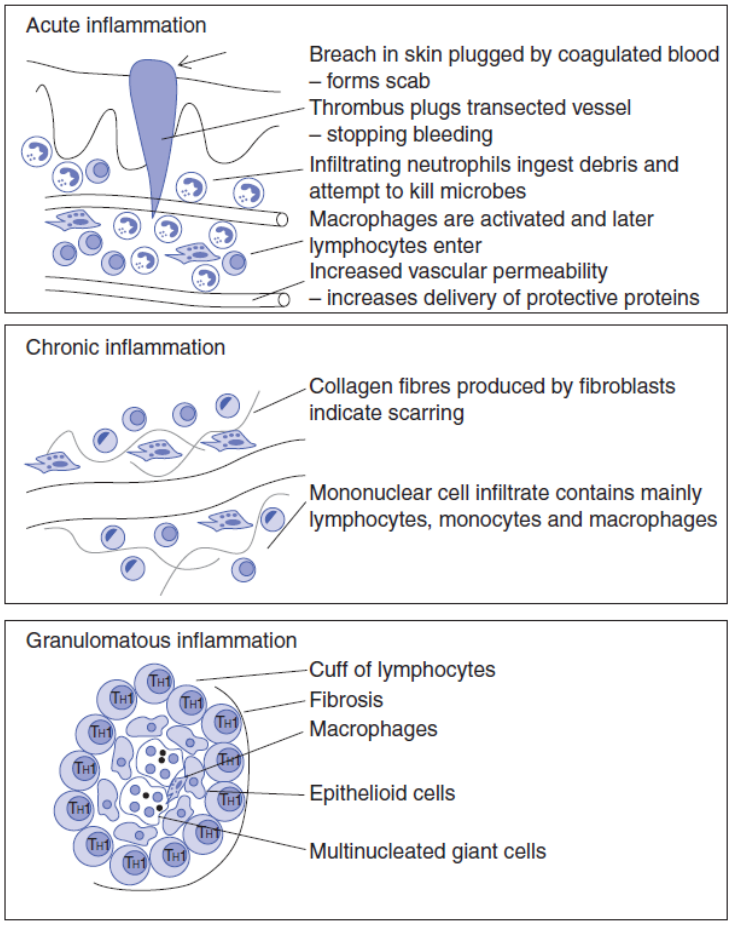

Figure 3.24 Common types of inflammation.

Chronic inflammation is usually associated with impairment of function. Resolution is usually associated with fibrosis and scarring. When chronic inflammation cannot be fully controlled, progressive fibrosis may occur in association with progressive loss of organ function.

GRANULOMATOUS INFLAMMATION. Granulomatous inflammation is a special type of chronic inflammation associated with intense macrophage activation, driven by IFN-y producing helper T cells.

Macrophages differentiate into epithelioid cells with enhanced secretory function, and diminished phagocytic capacity. Epithelioid cells fuse into multi-nucleated giant cells. This collection of epithelioid cells, giant cells and lymphocytes is called a granuloma (Figure 1.7.3). Granulomatous inflammation is associated with infections with organisms such as Mycobacteria, Treponema pallidum (which causes syphilis) and fungi. Granulomatous inflammation also occurs around foreign bodies, in some autoimmune disorders and idiopathic conditions such as sarcoidosis.

IMMUNE RESPONSES TO INFECTION

VIRAL INFECTION. Viruses enter the body, gaining access to cells following adhesion to the cell surface. Once in the cell cytoplasm, the virus hijacks the host cells' protein synthesis machinery to produce viral proteins. The virus then assembles particles that can bud from the cell surface, and infect more cells. Viruses have a short extracellular phase in their life cycle, however, usually the virus is in the cytoplasm. Viral proteins are subject to protein degradation by pro- teosomes, and similarly to self-peptides, viral peptides are transported to the endoplasmic reticulum, incorporated into MHC Class I molecules and displayed on the cell surface. Thus in order to cause infection, a virus must

- ♦Enter the body
- ♦Adhere to and enter host cells
- ♦Produce viable viruses capable of infecting other cells.

Viral infection of a cell may lead to cell lysis, syncytium formation and occasionally to tumour formation. The course of a viral infection and the host immune response will be considered using influenza as an example.

Influenza is spread by inhaled droplets, containing viable virus. The first challenge faced by the virus is to adhere to the respiratory epithelium. The host's first line of defence is the mucociliary elevator, which traps the virus in mucus, while cilia waft the mucus to the oropharynx to be expectorated or swallowed. If the host has previously encountered the same strain of influenza, neutralising antibodies, particularly IgA, present in respiratory secretions may prevent adhesion of the virus to respiratory epithelial cells.

Viral neuraminidase reduces the viscosity of mucus, and in the absence of antibodies may allow the virus to bind to and enter respiratory epithelial cells. Once the virus has entered the cell, Type I IFNs (IFN-α. and -β) are produced by infected cells. Influenza replicates very rapidly within the cell, however, replication is slowed down by these IFNs. Additionally, IFNs have a paracrine effect on neighbouring cells, inhibiting viral entry and reducing protein synthesis making it difficult for the infecting virus to replicate.

Some viruses cause a reduction in HLA Class I expression on the surface of cells in an attempt to become invisible to the hosts T cells. NK cells are normally held in check by the presence of host HLA Class I on cells, and reduced HLA Class I expression may lead to killing of the infected host cell by NK cells. IFNs also enhance NK cell activity. NK cells produce IFN-y, which activates T cells and biases differentiation towards a TH1 response.

Injury of respiratory epithelium causes an inflammatory response resulting in an influx of neutrophils, monocytes and T cells. Uptake of virus particles or proteins facilitates antigen presentation by resident dendritic cells or macrophages (APCs). In a primary immune response, the dendritic cells normally migrate to the draining lymph nodes. Intact antigen is also carried in lymph. Lymph node structure increases the chance of antigenbearing APCs meeting their cognate T and B cells. T cell activation leads to clonal expansion and differentiation of cytotoxic T cells and helper T cells. B cells are also activated and mature into plasma cells, producing antibody.

Antigen-specific T cells and antibody exit the lymph node in the blood stream. T cells activated by mucosal antigen are programmed to home to mucosal sites. Additionally, the presence of inflammation at the site of infection delivers more antibody and lymphocytes to the site. Peptide-specific cytotoxic T cells kill infected cells. Antibody may limit the ability of budding virus to enter other uninfected cells, and also plays a role in preventing future infection by the same virus.

Once the virally infected cells are killed, symptoms rapidly resolve and inflammation resolves slowly. It takes approximately 4 weeks for the ciliated respiratory epithelium to normalise completely, and during this time the host is more vulnerable to bacterial infection. This secondary bacterial infection causes death in vulnerable subjects during influenza epidemics (Figure 3.25).

BACTERIAL INFECTION. Bacteria must also bypass the body's natural defences to cause infection. This may result from ineffective mucociliary function, obstruction of urinary flow or breaches in skin integrity. Once natural defences are breached, bacteria usually remain in the extracellular compartment. The innate immune response includes activation of complement (by the alternative pathway). However, bacterial capsules inhibit complement deposition, allowing encapsulated bacteria to evade complement activity. Bacteria can be phagocytosed by neutrophils and macrophages, however, this system is inefficient in the absence of opsonisation. Natural opsonins such as C reactive protein and mannan binding lectin bind some bacteria. MBL activates complement via the lectin pathway and also independently opsonises organisms.

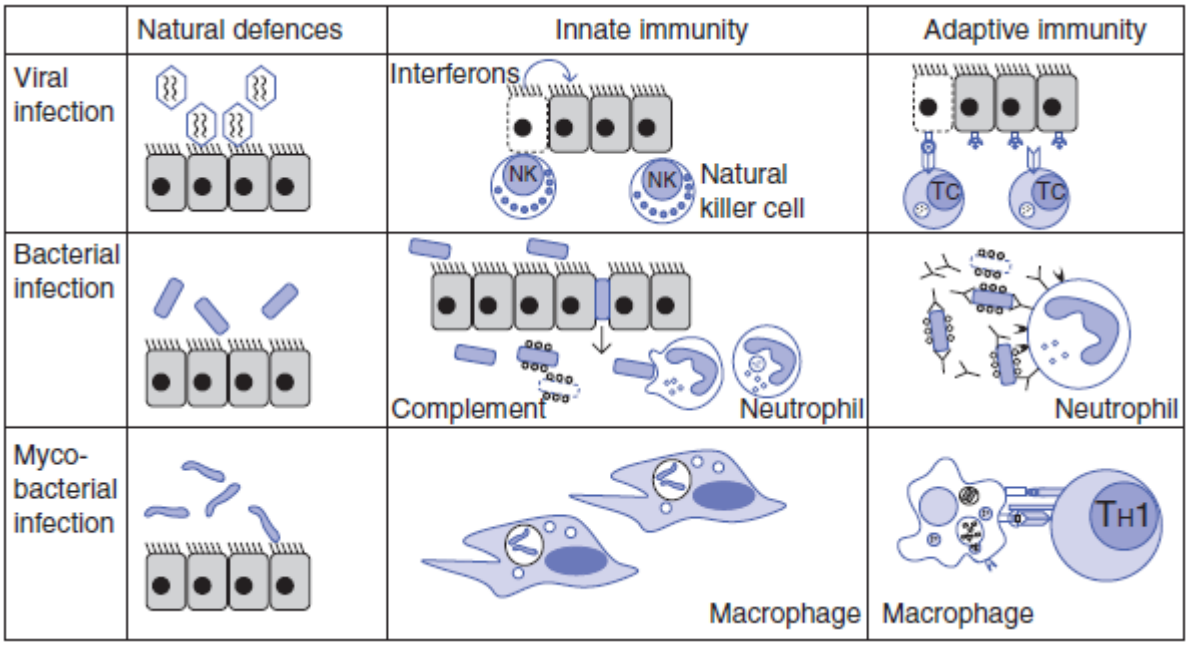

Figure 3.25 Overview of immune responses to infections.

As bacteria proliferate and die, bacterial antigens are released and carried in lymph to the lymph nodes. Intact antigen and APCs that have ingested antigen, travel to the draining lymph node. Both T and B cells are activated. T cell help is essential for B cells to undergo affinity maturation and isotype switching. Antibody may be produced locally or after plasma cells have migrated to the bone marrow. Antibody enters the blood stream, and delivery of antibody as well as complement and inflammatory cells is enhanced by inflammation at the site of infection.

Antibody binding to bacteria activates complement via the powerful classical pathway, and this may result in lysis of susceptible bacteria. Antibody also opsonises bacteria, facilitating bacterial ingestion by phagocytic cells. Antibody and complement act synergistically as opsonins. Ingestion of bacteria by healthy phagocytic cells results in bacterial killing and digestion.

Antibody reduces the risk of future infection by the same bacterium, as neutralising antibody prevents bacterial adhesion to cells, and preformed antibody may kill a small innoculum of bacteria before clinically apparent infection occurs.

MYCOBACTERIAL INFECTION. There are over 60 species of mycobacterium known, the majority of which do not cause disease in humans. Mycobacterium tuberculosis and Mycobacterium leprae cause tuberculosis (TB) and leprosy, respectively.

Usually M. tuberculosis enters the body via the respiratory tract, although gastrointestinal TB may also be seen. Once inhaled, mycobacteria may be cleared by the mucociliary elevator. However, if mycobacteria gain access to the lung, they are taken up by macrophages through recognition of mycobacterial lipoproteins by Toll-like receptors (TLRs). Within the phagosome the mycobacterium is relatively resistant to lysis because of a waxy protective capsule, as well as its ability to inhibit fusion of lysosomes with the phagosome. Failure of lysosome fusion prevents acidification of the phagosome and inhibits killing of the mycobacterium. Thus the innate immune response is relatively ineffective at killing M. tuberculosis. However, cells of the innate immune response play a pivotal role in activating the adaptive response.

Macrophages secrete IL-12 and present antigen, eliciting a TH1 response. TH1 cells produce IFN-y and TNF-α, further activating macrophages and enhancing the macrophages ability to kill mycobacteria. TH1 induced macrophage maturation results in granulomatous inflammation, and the infection is usually sealed off. The centre of granu- lomata may become hypoxic and undergo necrosis (termed “caseous necrosis”). It is common for some mycobacteria to persist for years or decades within granulomata, while

the host remains in good health. However, mycobacterial infection may become reactivated, usually within the lungs, if macrophage function is even moderately inhibited - for example by steroid treatment, malnutrition or other immunosuppressive therapy. More profound immunosuppression with TNF-α. blockade, HIV, or potent immunosuppression may allow widespread reactivation, which spreads beyond the lungs. This demonstrates that an on-going TH1 driven response is essential to maintain lifelong control of a tuberculous lesion.

In order to cause infection all organisms must bypass the body's natural defences. Once a pathogen enters the body, the innate immune response immediately aims to control an infection. The adaptive immune response is slower but more effective in eliminating infection. The type of pathogen determines which elements of the innate and adaptive immune responses contribute to sterilising immunity. Cells of the innate immune response frequently activate the adaptive response. Additionally, the adaptive immune response can harness many of the effector mechanisms of the innate response.

Theme №4. BASIC PRINCIPLES IMMUNOTROPIC DESTINATION THERAPY. IMMUNOREHABILITATION, IMMUNOPROPHYLAXIS

IMMUNOSUPPRESSION

The immune system plays a vital role in protection against infection and malignancy. Therefore, the potential benefits of treating a patient with immunosuppression must be assessed in the context of the associated risks. Not all patients with immunological diseases are treated with immunosuppression. Patients with apparently similar conditions may require different treatment regimens. Decisions on appropriate therapies may be based on evidence provided by controlled trials in common disorders. However, when treating uncommon disorders evidence is often lacking. Therapy may be based on knowledge of the immunological mechanisms of the disease and the mechanisms of action of immunosuppressive agents. The following units outline different immunosuppressive drugs in common use. This parts outlines the broad principles to consider when choosing an immunosuppressive regimen for an individual patient.

WHO REQUIRES IMMUNOSUPPRESSION? Patients with active, immune-mediated disease where suppression of the immune process is necessary to restore or improve health. The form of immunosuppression must be chosen on evidence of efficacy or likely efficacy, given the mechanism of action of the therapeutic agent and the pathogenesis of the condition to be treated. Criteria for assessing disease activity and monitoring of response to therapy are critically important. Therapeutic options other than immunosuppression may be preferable (e.g. hormone replacement in autoimmune endocrinopathies). Burnt-out disease where organ dysfunction is due to scarring will not respond to immunosuppression.

WHICH AGENTS? High quality, randomised controlled trials of different treatment regimens may provide evidence to guide therapy. However, where evidence is lacking knowledge of the underlying mechanism of disease and natural history of the condition directs treatment decisions.

Drugs are chosen to inhibit the appropriate part(s) of the immune system. Some immunosuppressive agents take weeks to produce therapeutic effects and are not adequate where organ damage progresses rapidly.

MINIMISING DOSE AND DURATION OF IMMUNOSUPPRESSION The risks of opportunistic infection and malignancy are related to both intensity and duration of immunosuppression. To minimise these risks the least toxic agent likely to be effective is chosen, the lowest dose likely to be effective is used, and powerful toxic agents such as cyclophosphamide are substituted with less toxic agents such as azathioprine as soon as possible. Dose reduction supervised by experienced clinicians

minimises the duration of immunosuppression. However, overzealous tapering of immunosuppression may result in relapse and exposure of patients to greater cumulative doses of drugs.

PATIENT MONITORING Patients are monitored for their response to therapy and anticipated side effects. Monitoring disease avoids over or under immunosuppression. Early detection and treatment of relapses may result in lower cumulative exposure to immunosuppression. Many immunosuppressive drugs have haematological, hepatic or renal side effects and monitoring for toxicity is required. Cyclosporin and tacrolimus levels are monitored in transplantation; however, levels are less helpful in other conditions where the optimal target levels remain to be established.

APPROPRIATE PROPHYLAXIS Many patients require long-term immunosuppression with regimens that are associated with predictable toxicity. Appropriate prophylaxis should be considered when therapy is being initiated. For example, PCP prophylaxis is indicated in patients requiring cyclophosphamide, and osteoporosis prophylaxis is routine in patients requiring chronic steroid therapy.

PATIENT EDUCATION Compliance with treatment is higher when patients understand their treatments and the reasons for taking each drug. Additionally, patients must be aware of side effects and understand the significance of febrile illnesses or possible infection. Patients need to be aware of the importance of reporting mouth ulcers or abnormal bleeding (early symptoms of leucopaenia or thrombocytopaenia) immediately when marrow suppression is a risk. Patients should also be aware of the importance of participation in screening programmes (e.g. cervical smears) as well as UV avoidance to minimise the risk of skin cancers.

CORTICOSTEROIDS

The structure and function of corticosteroids (steroids) resemble cortisol, a hormone secreted by the adrenal cortex. Cortisol (hydrocortisone) has many different actions, including mobilisation of glucose stores (glucocorticoid effects), maintenance of blood pressure control (mineralocorticoid effects) and suppression of inflammation. Cortisol protects the body during stressful events such as trauma, surgery and infection where baseline levels increase more than 10-fold.

Many different corticosteroids are used for their anti-inflammatory effects. Antiinflammatory activity is related to glucocorticoid effects and agents with a high ratio of glucocorticoid to mineralocorticoid activity are widely used in inflammatory diseases. Prednisolone is the most commonly used oral steroid, while hydrocortisone or methyl- prednisolone are given intravenously. Steroid agents are available for local administration to the skin, respiratory tract and gastrointestinal tract. Hydrocortisone is used for replacement therapy in adrenal and pituitary failure, where endogenous steroid production is inadequate.

INDICATIONS FOR USE Steroids are used to control inflammation in many different settings. These include:

♦Post-organ transplantation - prevention and treatment of organ rejection

♦Connective tissue diseases - for example, SLE; vasculitides and RA

♦Autoimmune diseases - immune haematological diseases - ITP and autoimmune haemolytic anaemia; chronic active hepatitis; pemphigus; some types of glomerulonephritis

♦Allergic inflammation - for example, asthma; atopic eczema; allergic rhinitis; acute allergic reactions

♦Inflammatory diseases - inflammatory bowel diseases; multiple sclerosis

♦Malignancies - for example, treatment of leukaemias and lymphomas; intracerebral pressure reduction in brain malignancies.

MECHANISMS OF ACTION The mechanisms by which steroids suppress inflammation include:

♦Monocyte effects - impaired maturation to macrophages. Reduced antigen uptake and processing. Inflammatory cytokine production (IL-1, IL-6, TNF-α) is diminished.

♦Lymphocyte effects - impaired cytokine production reducing proliferative responses and functional activity (one of the most important anti-inflammatory mechanisms of steroid therapy). Impaired recirculation causing lymphopaenia. High doses kill lymphocytes by apoptosis.

♦Prostaglandin inhibition - inhibition of COX enzymes and of membrane phospholipid release.

ADMINISTERING STEROIDS The dosage, route and duration of use of steroid medications are dependent on the clinical indication. Steroid therapy regimes are well established in many disease states and readers are referred to individual units for more information.

ADVERSE EFFECTS Prolonged use of steroids or use of high doses for more than a short time (7 days) exposes patients to many potential adverse effects. Side effects can be categorised into:

♦Exaggeration of physiological action of steroids

♦Suppression of endogenous steroid production with relative steroid insufficiency during stress.

STEROID PRESCRIBING Before initiating steroid therapy the following points should be noted:

♦Consider the benefit and risks of steroid therapy and plan therapy accordingly. Use a safe, effective, non-steroid option if available.

♦Consider objectives of therapy and plan dose and duration of treatment accordingly - the initial dose should achieve control and dose should be reduced to the lowest possible effective dose as rapidly as possible.

♦Local administration (e.g. airway inhalation, application to skin or rectum) is preferred to systemic administration if possible. The side-effect profile is much less severe than with systemic steroid therapies. However, high dose inhaled steroids may cause impaired growth in children.

♦Steroids are best administered in the morning, or on alternative days to limit effects on endogenous steroid production.

♦Agents are not interchangeable and have different anti-inflammatory potencies (strengths). An equivalency chart is available in the British National Formulary (BNF), however equivalence values are approximate and vary in different clinical situations.

♦Enteric-coated steroids may be erratically absorbed. In general, non-coated preparations are preferred if tolerated.

♦Gastric and bone protection should be considered in patients at risk of peptic ulceration or osteoporosis, particularly in patients requiring long-term therapy. Bone density monitoring is indicated in patients receiving long-term therapy.

♦Patients should be aware of increased steroid requirements during stress (which persists after cessation of steroid therapy), and the dangers of abrupt cessation of therapy. All healthcare providers should be aware of the history of steroid use.

♦Vigilance regarding infection, especially chicken pox and tuberculosis (TB) is important for patients and doctors.

♦Patients should be advised to carry a ‘steroid user card’ or an alert pendant to provide information in an emergency.

The drugs outlined in this section work primarily by inhibiting proliferation of lymphocytes. These agents affect T cell responses and B cell proliferation to varying degrees. Many of the side effects are due to anti-proliferative effects on other rapidly dividing cells in the body. The main differences are in potency and toxicity.

AZATHIOPRINE

First synthesised in 1961, this agent is widely used in transplantation and autoimmune disease, where it may allow substantial reduction of steroid dosage. The parent drug is inactive until metabolized in the liver to 6-mercaptopurine (6-MP). It is a moderately potent alkylating agent, whose major

mechanism of action is inhibiting conversion of inosine to adenosine, leading to cellular adenosine depletion and impaired proliferation.

Azathioprine is usually administered orally. Many side effects of azathioprine are due to non-specific inhibition of proliferation, especially in the bone marrow. Patient education and monitoring of full blood count (FBC) and liver function tests allow early detection of dose-related bone marrow suppression and idiosyncratic hepatotoxicity. Other side effects include nausea, hypersensitivity reactions and hair loss. There is an increased susceptibity to infection and increased risk of malignancy, especially skin cancer. If severe nausea precludes the use of azathioprine, 6-MP may be tolerated.

METHOTREXATE

Methotrexate (MTX) interferes with DNA synthesis by blocking a critical folate pathway enzyme. It inhibits cell proliferation. It is used in large doses for the treatment of malignant conditions including some leukaemias. Bone marrow suppression, liver dysfunction and mucositis are common adverse side effects. Moderate doses given either by the oral or intramuscular routes are used with benefit in a number of immune-mediated conditions including rheumatoid disease and severe psoriasis. Gradual dose elevation from an initially small

dose is undertaken once tolerance is ensured. It is important to note that MTX used in these settings is administered once weekly rather than daily. Inadvertent prescribing errors are well documented and result in critical bone marrow suppression and often death. Such unfortunate cases require admission to units where expert management of the bone marrow aplasia can be given. Folinic acid is given as a rescue treatment and works by by-passing the metabolic step inhibited by MTX.

All patients receiving MTX must be aware of the potential side effects and their likely presentations. Liver and pulmonary adverse effects are well documented in addition to the bone marrow effects. Careful and regular monitoring of the FBC and liver function tests (LFTs) is mandatory. Patients should be advised not to drink alcohol while taking this drug. MTX is associated with development of a pneumonitis which is an indication for immediate cessation. Before treatment, formal pulmonary function assessment is performed. Regular clinical and functional assessments allow early detection of pneumonitis.

MYCOPHENYLATE MOFETIL

Mycophenylate mofetil (MMF) has recently been licensed for prophylaxis of acute renal and cardiac transplant rejection. MMF is a specific, non-competitive inhibitor of inosine monophosphate dehydrogenase, a key enzyme in the de novo pathway of purine synthesis. Lymphocytes are highly dependent on the de novo pathway, and unlike most other cells cannot utilise the salvage pathway (which allows recycling of purine bases). Therefore, the anti-proliferative effect of MMF shows some lymphocyte specificity.

Dose-related gastrointestinal intolerance is common. Potential bone marrow suppression requires FBC monitoring, as well as patient education to recognise signs of possible bone marrow suppression (infections, fevers, mouth ulcers, bruising or unexplained bleeding). Infection is more commonly seen than with azathioprine, and increased incidence of malignancy is also reported. Hypersensitivity reactions and many other side effects have been reported.

The therapeutic effect of MMF appears superior to azathioprine, particularly in suppression of humoral immune responses. Although this agent is not yet licensed for use in autoimmune disease, many trials and case reports describing successful use in SLE and other antibody-mediated disorders have been published.

CYCLOPHOSPHAMIDE

Cyclophosphamide (CP) is an alkylating agent, which is widely used in cancer treatment. At lower doses it is used as a potent, but toxic immunosuppressive drug. CP cross-links DNA, inhibiting cell proliferation. This effect is not lymphocyte-specific and severe toxicity may be seen in other rapidly

dividing cells. Its use is generally reserved for life-threatening or organ-threatening immunological diseases.

Administration is by a daily oral dose or pulse IV doses usually given monthly. Dose reduction may be needed in the elderly, or in severe renal or hepatic impairment.

Bone marrow suppression is commonly seen. Regular monitoring together with patient education reduce the associated risks. Haemorrhagic cystitis results from irritation of bladder epithelium by a CP metabolite, acrolein. Bladder protection by hydration or use of a drug called mesna reduces the risk of haemorrhagic cystitis. CP is generally less hepatotoxic than azathioprine, however alopaecia is seen more commonly. Prolonged treatment with CP may lead to both male and female infertility. Susceptibility to infection is marked, and prophylaxis against PCP is recommended. CP carries a significant risk of secondary cancers developing several years following therapy (Figure 4. 1.)

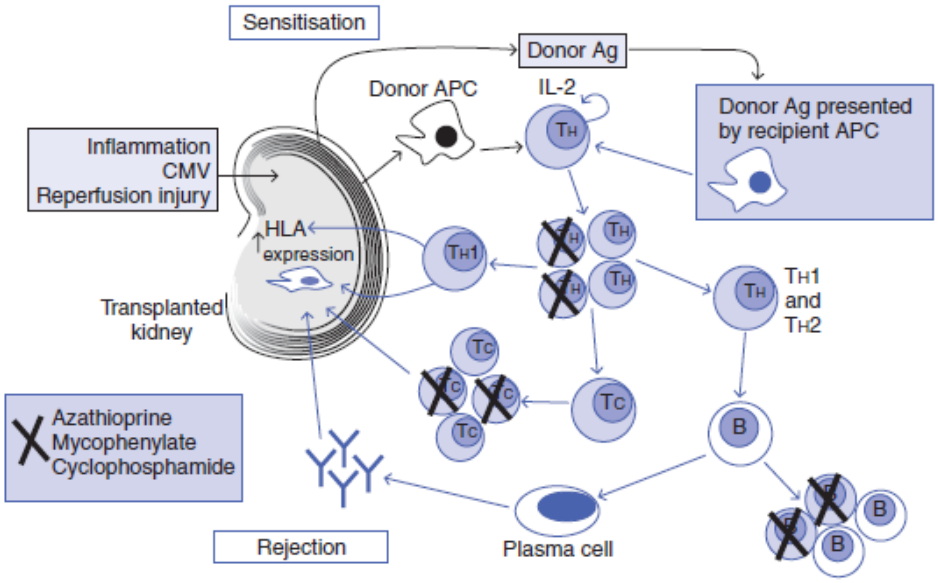

Figure 4. 1 Immunosuppression: anti-proliferative mechanism of action of azathioprine, mycophenylate and cyclophosphamide.

The agents described in this section suppress T cell responses predominantly. The oldest agent cyclosporin A, introduced in 1980, dramatically improved the results of renal transplantation and allowed the development of liver, heart and lung transplantation.

CYCLOSPORIN

Cyclosporin binds cyclophilin and inhibits activation of T lymphocytes by preventing the production of IL-2 and some other early T cell activation genes. It is a lipid-soluble drug, metabolised in the liver by CYP3A4. Cyclosporin is widely used as part of triple therapy (with steroids and azathioprine or mycophenylate) in solid organ transplantation. It is also used to prevent GvHD following bone marrow transplantation, for severe resistant atopic dermatitis and asthma, aplastic anaemia and occasionally in severe autoimmune disorders.

Two preparations are available - Sandimmune and Neoral. These are not interchangeable due to significant differences in bioavailability. In transplantation cyclosporin dosage is monitored by drug level measurement. Traditionally, this has been based on the trough level, however a 2-hour peak level may be preferable. In autoimmunity, the appropriate target level has not been defined, and so monitoring is aimed at preventing nephrotoxicity. Blood pressure and creatinine levels are maintained within predefined limits (usually creatinine should remain within 30% of pre-treatment values).

Cyclosporin interacts with many drugs, including over the counter therapies and herbal remedies. Patients must be warned about this and monitoring increased when additional therapies are required. The

most serious side effect is nephropathy, which causes renal failure in over 10% of recipients of non-renal solid organ transplants. Other common side effects include hypertension, infection, impaired glucose metabolism, tremor, gastrointestinal (GI) disturbances, gingival hypertrophy, hirsutism, malignancies and neurological disorders.

TACROLIMUS

Tacrolimus (FK506) binds to FKBP-12 (FK binding protein-12) in cells, and inhibits T cell activation by a mechanism identical to cyclosporin. Absorption is bile-independent. Acute however graft survival appears to be improved only in liver and intestinal transplants. Use of tacrolimus in autoimmune disease has not been extensively studied. Topical tacrolimus has recently become available for treatment of moderate to severe atopic dermatitis (AD). The side effect profile is similar to cyclosporin with nephropathy being a major problem. Diabetogenic potential may be greater than cyclosporin, however gingival hypertrophy and hirsutism do not occur. Dosage of tacrolimus is monitored by drug-level measurement (Figure 4.2.)

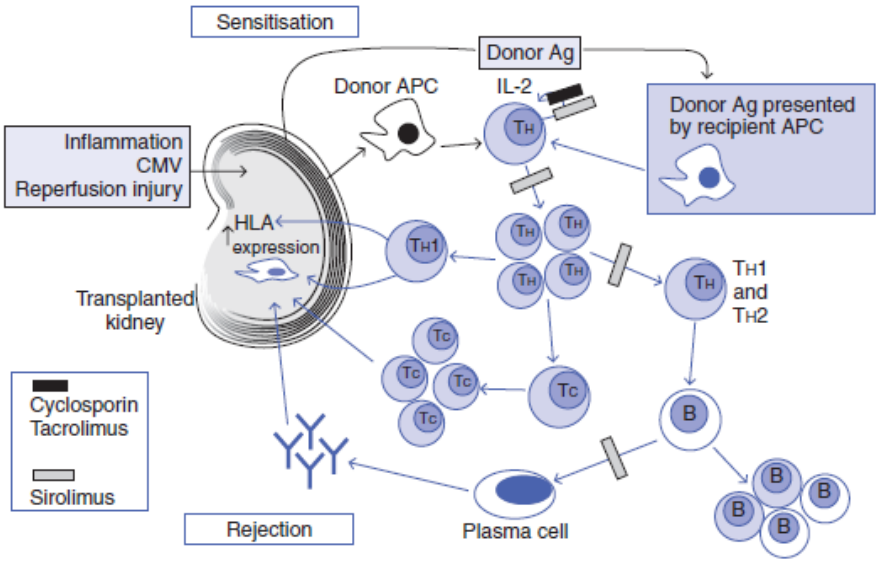

Figure 4.2 Immunosuppression: mechanism of action of cyclosporin, tacrolimus and sirolimus.

RAPAMYCIN (SIROLIMUS)

Rapamycin binds to TOR (target of rapamycin) and this complex binds FKBP-12, however the mechanism of action is distinct from Tacrolimus. Rapamycin inhibits cytokine-mediated signalling in late G1 phase, and blocks the action of IL-2, IL-4 and IL-6. Rapamycin is a potent inhibitor of T cell responses, but also inhibits B cell responses and smooth muscle proliferation (potentially important in chronic rejection). Rapamycin is used in solid organ transplantation.

Rapamycin absorption is impaired by fatty food, and the drug is metabolised in the liver by CYP3A4. Because of this there is a clinically significant interaction with cyclosporin. Rapamycin dosage is guided by drug levels. Rapamycin is not nephrotoxic. Principal side effects include severe arthralgia, hyperlipidaemia and impaired wound healing.

Of interest, rapamycin does not inhibit induction of tolerance in animal studies, whereas cyclosporin and tacrolimus do. In the future, this may become an important property.

THERAPEUTIC ANTIBODY PRODUCTION

Antibody-based therapies are increasingly used in a growing spectrum of clinical conditions. Both polyclonal and monoclonal antibodies are used therapeutically. Antibodies raised in other species are foreign proteins, and may produce an antibody response when administered therapeutically. Immunogenicity has limited the repeated use of these agents in chronic disease. In the last decade, reshaped antibodies have been produced, in an attempt to limit immunogenicity. Principles of antibody

production are described in this section; subsequent sections describe routine use of some therapeutic antibodies.

Therapeutic uses of antibodies include:

♦Prevention of infectious disease

♦Neutralisation of toxins

♦Prevention of rhesus alloimmunisation

♦Immunomodulation

♦Treatment of malignancy

♦T cell depletion of bone marrow for transplantation.

POLYCLONAL ANTIBODIES

Most antigens stimulate multiple lymphocytes as part of the normal immune response. Antibodies produced in such responses are polyclonal. Well-established techniques are available for isolation and purification of specific antibodies from plasma. Therapeutic antibodies may be purified from human plasma or raised in animals (usually rabbits or horses). There are stringent manufacturing requirements to ensure quality, purity and sterility of antibodies produced for therapeutic use.

Therapeutic polyclonal antibodies of human origin include:

♦ Concentrated preparations of rhesus antibodies, used to prevent rhesus alloimmunisation

♦Pathogen-specific antibody concentrates (hyper-immune globulins) used as post-exposure prophylaxis (e.g. Varicella-Zoster and Hepatitis B).

Therapeutic polyclonal antibodies of animal origin include:

♦Anti-thymocyte globulin (ATG) used in solid organ transplant rejection therapy

♦Tetanus immune globulin for neutralisation of tetanus toxin.

MONOCLONAL ANTIBODIES (MAbs)

Besides antibody- based therapeutic agents, MAbs have also revolutionised laboratory procedures. The importance of MAbs is reflected in the award of a Nobel Prize to Kohler and Milstein, the scientists who described the technique of MAb production in 1975.

The major advantages of MAbs over polyclonal antibodies include:

♦Production quantities - near-limitless amounts of antibody can be produced with stable activity over long periods of time.

♦Specificity - antibody specificity remains stable. Monoclonal does not necessarily imply monospecificity. However, high-affinity antibodies without clinically relevant cross-reactivities are chosen as therapeutic agents.

♦Consistency - batch-to-batch variation is rarely seen, unlike polyclonal antibody preparations.

Theoretically, MAbs could be generated using cells from any animal, however rodents are the only species in which hybridomas are produced with a high success rate. The overall structure of mouse immunoglobulin is similar, but not identical, to human immunoglobulin. This poses two problems in relation to in vivo use.

♦Adverse reactions - antibody production against ‘foreign’ components can cause systemic reactions of varying severity. Fc effector components of MAbs may also trigger generalised immune activation with similar effects.

♦Loss of efficacy - ‘anti-antibodies’ induced against species-specific components of MAbs can also impair antibody persistence and function with reducing efficacy with repeated use.

RESHAPED MAbs

Production of human MAbs is technically challenging but continues to be a goal. However, using DNA technology, chimeric and humanised MAbs have been produced and used as therapeutic agents (Figure 4.3).

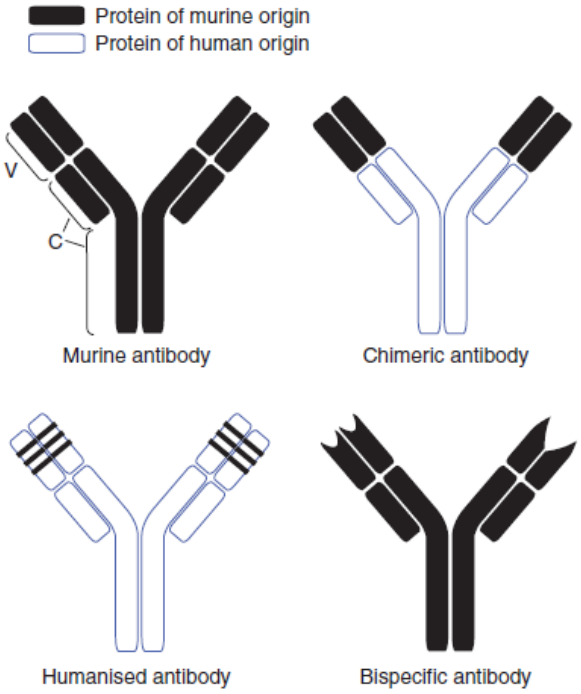

Figure 4.3 Comparison of MAbs, chimeric MAbs, humanised MAbs and bispecific antibodies.

Chimeric antibodies are produced by introducing the gene segments encoding the variable parts of both the heavy and light chains of the rodent MAb of interest into the corresponding human immunoglobulin genes. The resulting antibody is predominantly human, but still contains a significant amount of rodent protein. Approximately, 10% of human recipients of such antibodies will make human-anti-chimeric antibodies (HACAs). HACAs may inhibit antibody function. Development of antibodies is less common in patients who are receiving concomitant immunosuppression. Serum sickness reactions are rarely problematic.

Humanised antibodies were developed to further decrease the amount of rodent protein included in therapeutic antibodies. These antibodies are produced by grafting the hypervariable regions of both heavy and light chains onto the framework of a human immunoglobulin gene.

TARGETED CELL DEPLETION Several biological reagents have been developed in an attempt to deplete specific cells of the immune system, to facilitate more potent or more specific immunosuppression. Heterologous polyclonal antibodies, heterologous monoclonal antibodies and humanised monoclonal antibodies are in routine use.

A common side effect of all depleting antibodies is the cytokine release syndrome (CRS). This occurs as a result of cytokine release from activated lymphocytes and monocytes. Typical clinical features include fevers, chills and rigors, rash, musculoskeletal pain, bron- chospasm, dyspnoea and hypotension. CRS is usually most severe after the first infusion, becoming less severe with subsequent infusions.

ANTI-THYMOCYTE GLOBULIN

Anti-thymocyte globulin (ATG) is a polyclonal heterologous antiserum, produced by immunising rabbits or horses with human T cells or a human T cell line. It is used in combination with other immunosuppressive drugs to prevent organ rejection and aplastic anaemia. In solid organ transplantation, ATG is used as prophylaxis in high-risk patients, or to treat steroid-resistant rejection.

Since ATG is a polyclonal product, there are differences between preparations, and even different batches from the same manufacturer. ATG depletes cells both by cytotoxicity and opsonisation, which results in removal of antibody-coated cells by the reticulo-endothelial system. Additionally, by binding to a number of cell surface molecules, ATG may inhibit function of the residual cells. Effectiveness may be monitored by measuring absolute T cell counts.

ATG is diluted and infused into a large vessel over several hours. Premedication with steroids and antihistamines reduces the incidence of systemic side effects. ATG is contraindicated in patients with allergy to rabbit (or horse) proteins, severe thrombocytopaenia, active infection or during pregnancy. Prior to administration, a skin test is used to rule out allergy to the heterologous antiserum. Side effects include CRS, anaphylactic reactions (Type I hypersensitivity) and serum sickness after 8-14 days (Type III hypersensitivity). If a second course of ATG is required, antiserum from an alternative species should be chosen. If a second course is given from the same species there is a high risk of hypersensitivity and skin test results must be closely examined. The progress of T cell depletion, which can be impaired by neutralising antibodies, should be monitored.

MUROMONAB-CD3 (OKT3)

Muromonab-CD3 (commonly known as OKT3) is a murine monoclonal antibody against CD3, used to treat acute rejection (usually steroid-resistant rejection) of renal, hepatic and cardiac transplant patients.

Muromonab-CD3 results in depletion of T cells, leading to marked inhibition of cellular immune responses. Established B cell responses are not affected, however T cell help for

B cell maturation is inhibited. The effectiveness of treatment can be monitored using absolute T cell counts.

Muromonab-CD3 is administered as a daily bolus injection for 10-14 days. Methylprednisolone is given prior to the first dose to reduce the incidence and severity of the CRS, which occurs in most patients after the first dose.

Muromonab-CD3 is contraindicated in patients who are hypersensitive to mouse products, have anti-mouse titres of >1: 1000, uncompensated heart failure or fluid overload, a history of seizures or in patients who are pregnant/breastfeeding.

Side-effects of muromonab-CD3 include anaphylaxis which may be difficult to differentiate from CRS, neuro-psychiatric events as well as complications of immunosuppression (infection, neoplasia and viral-induced lymphoproliferative disorders). Repeated treatment with muromonab-CD3 carries a risk of allergic reactions and inhibition by neutralising antibodies.

RITUXIMAB (ANTI-CD20)

Rituximab is a humanised anti-CD20 monoclonal antibody licensed for the treatment of chemotherapy-resistant follicular lymphoma. It has also been used in some antibody-mediated autoimmune diseases.

Rituximab is a cytotoxic antibody that binds to and depletes CD20-positive B cells. Plasma cells are CD20 negative, and are not depleted. Rituximab inhibits B cell maturation and may reduce autoantibody levels. Cellular immunity is not directly affected. However, B cells can present antigen to activated or memory T cells, so an indirect effect appears likely.

Rituximab is administered as an infusion, once weekly for 4 weeks. An analgesic and antihistamine, with corticosteroids are given pre-infusion. Repeated courses of treatment can be given, as humanisation of antibody reduces immunogenicity.

Side effects include infusion reactions (including CRS) and leucopaenia. Tumour pain and tumour lysis syndrome may also occur in patients with a high tumour burden.

CAMPATH-1H (ANTI-CD52)

Campath-1H is a recombinant humanised monoclonal antibody directed against CD52, a cell surface molecule, expressed on T and B cells and at low levels on NK cells. It is licensed for treatment of resistant B cell chronic lymphocytic leukaemia (B-CLL). Campath-1H has also been used for other lymphoid malignancies, autoimmune cytopaenias, RA, and induction therapy for renal transplantation.

Campath-1H depletes T and B cells in vivo and in vitro, inhibiting both cellular and humoral responses. B cell depletion lasts several months and T cell cytopaenia, persists for over a year. NK cell numbers fall temporarily, but usually recover in weeks.

Campath-1H is given as an intravenous infusion, usually after premedication with paracetamol and an antihistamine. Prophylaxis against herpes infections and Pneumocystis carinii is recommended.

Infusion-related side effects are commonly seen and are typical of CRS. Prolonged haematological toxicity and infections also occur.

Campath-1H is also used in vitro to T cell deplete bone marrow and other sources of stem cells. (Figure 4.4)

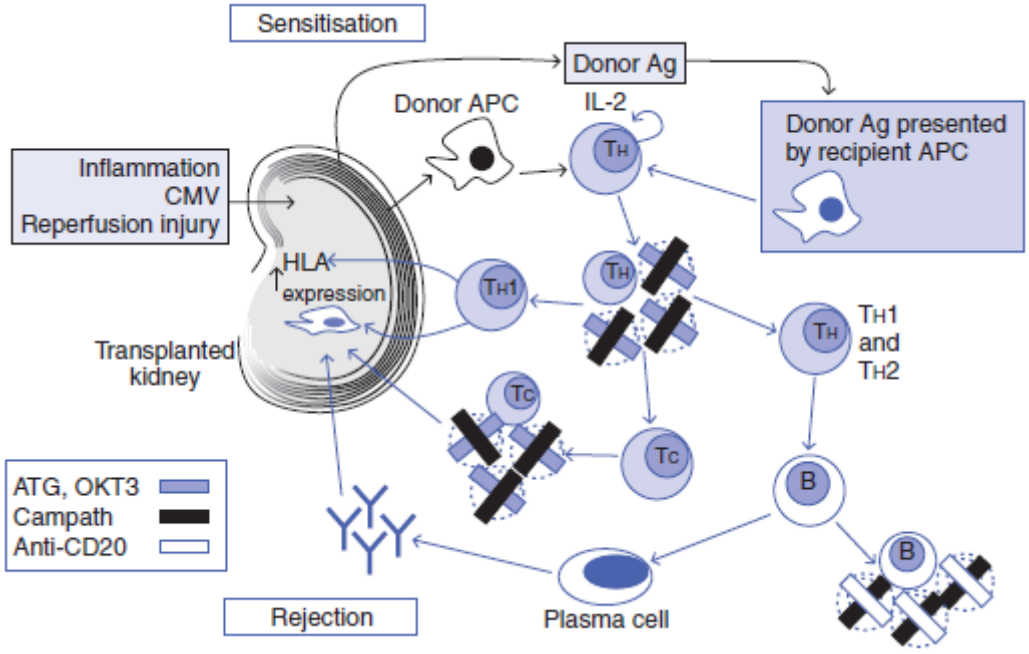

Figure 4.4 Immunosuppression: ATG, OKT3, Campath and anti-CD20.

NEW AGENTS

New depleting antibodies that target specific cell populations in the immune system are under development, including antibodies to CD25, which are specific for activated lymphocytes. Additionally, non-immunological applications of targeted cell depletion include antibody-induced eradication of breast cancer cells that overexpress a growth factor receptor called herseptin.

IMMUNOGLOBULIN REPLACEMENT THERAPY

Immunoglobulin (Ig) concentrates are prepared from pooled plasma donations from 1000-10,000 individuals. Careful donor screening, strict regulation and quality control of production, and additional anti-viral treatment of Ig concentrate, produces a very safe product. Ig contains predominantly IgG with variable levels of IgA contamination. A number of products are commercially available. Early preparations were for intramuscular administration (IMIg). Dosage was limited by pain at injection sites and side effects were common. Products suitable for intravenous use (IVIg) available since the 1980s are now widely used. Products for subcutaneous administration are also available. Ig prepared for different routes of administration, and even different IVIg products are not interchangeable. Ig is extremely expensive and supply is limited worldwide. Therefore, it is critical that its use is reserved for cases where it is clearly indicated. Clinical immunologists should be consulted prior to use outside the indications outlined in the following paragraph.

INDICATIONS FOR IVIg THERAPY

Primary antibody deficiency syndromes IVIg is recommended therapy in CVID, XLA, CD40L deficiency, other severe genetic antibody deficiencies. Its value in IgG subclass deficiency and specific antibody deficiency is less clear. Trial use for a 12-month period may be useful. Temporary use in symptomatic transient hypogammaglobulinaemia of infancy may be useful.

Secondary antibody deficiency syndromes IVIg is of value in patients with antibody deficiency associated with multiple myeloma and chronic lymphocytic leukaemia. Benefit of IVIg is most marked in those patients with a history of pyogenic infection or where low specific antibody levels and poor vaccine responses are demonstrated. IVIg is also used following bone marrow transplantation until immune reconstitution occurs.

T cell mediated immunodeficiencies IVIg is indicated in SCID and CID syndromes where impaired antibody-mediated immunity is demonstrated.

ADMINISTRATION Large doses of IVIg can be administered and normal levels of IgG achieved. Adverse reactions with IVIg are rare, provided care is taken with patient preparation and infusion. The average dose for replacement purposes is 0.4 g/kg every 3 weeks (half-life of IgG is 21 days). Doses and/or frequency of infusion are increased if patients do not reach normal levels of IgG after approximately 3 months of treatment. The IVIg requirement may be increased if infection is inadequately treated, with co-existent protein-losing states and rarely with hypercatabolism of Ig. Protein-losing states without associated immunodeficiency rarely require Ig replacement, despite low IgG levels. Subcutaneous administration of Ig can be useful where venous access is a problem. Intramuscular Ig is no longer used.

Initial therapy should be undertaken in a hospital setting, supervised by personnel familiar with IVIg therapy. Home therapy is an option for some patients and is organised by specialist centres that select, prepare and support patients.

Patient screening is required prior to first infusion. This includes:

- ♦Pre-infusion IgG level
- ♦Hepatitis virus screen (especially HCV)
- ♦Renal and liver biochemistry
- ♦FBC
- ♦Infection screen
- ♦Serum storage archive for ‘look-back’ studies if adverse events arise (e.g. infection transmission
- ♦Written, informed consent covering administration, side effects and potential long-term risks of use of this blood product.

ADVERSE REACTIONS Acute adverse reactions are most common with the first infusion of a particular IVIg product and are rare once treatment is established. Mild reactions cause headache, shivering, muscle aching and fever with severe reactions characterised by respiratory embarrassment, chest pain, hypotension, collapse and occasionally death. Symptoms occur during or within 24 hours of infusion and are usually due to immune complex formation. Causes include too rapid an infusion rate or untreated infection.

Guidelines to lessen the risk of reaction include:

- ♦Initiate infusions at rates slower than manufacturers’ recommendations
- ♦Adequate treatment of infection prior to infusion
- ♦Administer hydrocortisone and antihistamine before the first few infusions
- ♦Do not change products unless absolutely essential.

Most reactions settle on slowing or stopping the infusion. Paracetamol is helpful for mild reactions. Antihistamine and adrenaline may be required for more severe reactions.

Antibody-deficient patients with complete IgA deficiency may develop anti-IgA antibodies on exposure to IVIg-containing IgA, which causes adverse effects. Products depleted of IgA are preferable for such patients.

Occasionally, patients continue to have reactions despite addressing these issues. Changing to another product may be helpful. Occasionally, continued prophylaxis with paracetamol, antihistamine or even steroid is required.

No blood product is completely safe and transmission of blood-borne infection is a concern. Additional virucidal steps in IVIg production minimise this risk, however vigilance is always required. HIV infection has never been transmitted by IVIg. Hepatitis C virus has been transmitted by IVIg with serious consequences. Prion transmission risk (new variant Creutzfeld Jacob disease agent) is unknown but is possible in theory.

Attempts to limit the risk of blood-borne prion disease have seriously compromised the availability of plasma worldwide. Shortfalls in relation to demands are now regularly experienced posing life-threatening risks for antibody-deficient patients should supply fail.

PATIENT MONITORING

Patients receiving Ig replacement therapy should be monitored in relation to the following:

Efficacy

♦Clinical status (e.g. daily diary detailing well-being, infection, antibiotic usage).

♦Assessment of progress of underlying diseases - for example, pulmonary function and high resolution CT of thorax.

♦Trough levels of IgG (just before infusion) should be measured regularly and dose/interval adjusted accordingly. Trough IgG should be within the normal range and

probably higher in patients with established lung disease and in CVID patients with granulomata. Clinical status rather than IgG levels are the mainstay of monitoring of patients with multiple myeloma and CLL.

Safety

♦All infusions, product and batch numbers should be logged.

♦Interchange of product and batch number exposure should be minimised in individual patients.

♦Serial pre-infusion liver function testing and storage of samples for viral studies will allow prompt identification of infection should it occur.

♦Careful records facilitate identification of the culprit product.

CYTOKINES

Cytokines are soluble messengers, which coordinate the immune response. Physiologically, cytokines usually act in a paracrine fashion (exerting their action on nearby cells). Cytokines have many potent effects on the immune system, necessitating tight control of cytokine production during an immune response. At present, therapeutic use of cytokines cannot replicate this localisation or control of activity. Cytokines are small biologically active proteins; they cannot be administered orally due to local effects on the gut and digestion.

Despite limitations imposed by current delivery systems, cytokines are being used to treat a number of conditions:

♦Replacement therapy in cytokine deficiency

♦To augment or redirect the immune response

♦As immunomodulatory therapy.

Because of their generally short half-life, these agents often require frequent administration and/or modification of the molecule to increase the half-life.

REPLACEMENT THERAPY

IL-2 deficiency is a rare cause of SCID. Regular administration of IL-2 has been associated with clinical improvement and normalisation of T cell proliferation.

Genetically transmitted IL-12 and IL-12 receptor deficiencies and defects in the IFN-y receptor are associated with increased susceptibility to mycobacterial infections and salmonellosis. Regular treatment with subcutaneous IFN-y can be used as an adjuvant to treatment of infection as well as prophylaxis against further infections. In patients with partial deficiency of the IFN-y receptor high doses of IFN-y

may be effective, although not surprisingly such treatment is usually unsuccessful in complete deficiency of the IFN-γ receptor.

AUGMENTING THE IMMUNE RESPONSE

Cytokines are used in the treatment of cancers and some infections, where at least part of their therapeutic effect results from augmentation of the immune response. IL-2 is thought to have an anti-cancer effect due to immune stimulation. IFN-α has anti-tumour effects, which may be direct or related to the immunological effects of this cytokine. Interferons have also been used in a number of chronic infections. Beneficial effects are likely to be due to both immune stimulation as well as direct anti-viral effects.

Recombinant IL-2 is licensed for subcutaneous use in patients with metastatic renal cell carcinoma. It is highly toxic, and although tumour shrinkage has been documented, survival does not appear to be increased. Toxicity is universal and often severe with capillary leak syndrome, which causes pulmonary oedema and hypotension, as well as bone marrow, hepatic, renal and CNS toxicity.

IFN-α is used for treatment of haematological malignancies and solid tumours. Additionally, IFN-α is used as an adjuvant therapy for malignant melanoma as well as maintenance therapy for multiple myeloma in remission. Common side effects are dose-related and include nausea, influenza-like symptoms, lethargy and depression. Myelosuppression, cardiovascular problems, nephrotoxicity and hepatotoxicity may also occur.

IFN-α is also licensed for treatment of chronic Hepatitis B and Hepatitis C. In combination with ribavirin, IFN-α leads to clearance of virus in a significant proportion of patients with Hepatitis C. A beneficial effect of IFN-α has also been shown in lepromatous leprosy (where a predominantly T helper cell, type 2 (TH2) type response fails to control the infection), and in visceral leischmaniasis.

IMMUNOMODULATION

The original rationale for treating MS with IFN-β was based on the hypothesis that MS was due to a defective immune response to an unidentified viral pathogen. IFN-β was found to be beneficial in several types of MS. While the precise mechanism leading to clinical benefit in MS is poorly understood, the therapeutic effect of IFN-β is thought to be due to immunomodulatory effects. IFN-β rapidly restores the blood brain barrier in addition to inhibiting T cell proliferation, antigen presentation and T cell migration. Additionally, it appears to modify cytokine production towards an anti-inflammatory profile, both in the periphery and in the CNS.

IFN-β is self-administered by subcutaneous injection three times a week. The most common side effects are injection site reactions, influenza-like symptoms and depression.

The possibility of modulating or redirecting the immune response in a number of other immunological disorders using cytokines is currently under investigation, and the list of therapeutic applications of such therapies is likely to grow substantially over the next decade.

VACCINATION AND PASSIVE IMMUNISATION

Vaccination is the greatest success of immunology. In 1798 Jenner introduced a cowpox vaccine that protected against the antigenically related, lethal smallpox. Development of safe vaccines and mass vaccination led to the worldwide elimination of smallpox in the 1980s. The WHO aims to eliminate polio in the near future. Immunization is a means of providing specific protection against many common and damaging pathogens by stimulating an organism's immune system to either produce humoral antibodies against the pathogen (or toxins produced by the pathogen) or T cells that can provide cell-mediated immunity.

The type of immunity that is needed to neutralize a specific pathogen depends on the site of the pathogen and the mechanism of its pathogenesis. For example, some pathogens produce disease by secreting exotoxins. If this is the case, the only immune mechanism effective against the organism would

be neutralizing antibodies that prevent exotoxin binding to the appropriate receptor on its target cell and promoting its clearance and degradation by phagocytes.

If the pathogen produces disease by other means, an antibody will have to react with the pathogen itself and eliminate it either by complement-mediated lysis or phagocytosis and intracellular killing. However, if the pathogenic organism is localized intracellularly, it will not be accessible to antibodies and the cell harboring it will have to be destroyed instead; only then could antibody have any effect on the pathogen. Most viruses, together with intracellular bacteria and protozoa, are examples of such pathogens. In this case, the harboring cells can be destroyed by elements of cell-mediated immunity or, if they cause the infected cell to express unique antigens recognizable by antibody, antibody-dependent and complement-mediated killing of the infected cell can expose the pathogen to elements of humoral immunity. It is also possible for cells harboring intracellular pathogen to be activated to kill the pathogen. Such is clearly not the case with pathogens that have the capability of surviving within phagocytic cells.

Specific immunity can result from either passive or active immunization and both modes of immunization can occur by natural or artificial processes.

PASSIVE IMMUNITY

Immunity can be acquired, without the immune system being challenged with an antigen. This is done by transfer of serum or gamma-globulins from an immune donor to a non-immune individual. Alternatively, immune cells from an immunized individual may be used to transfer immunity. Passive immunity may be acquired naturally or artificially.

Naturally acquired passive immunity. Immunity is transferred from mother to fetus through placental transfer of IgG or colostral transfer of IgA.

Artificially acquired passive immunity. Immunity is often artificially transferred by injection with gamma-globulins from other individuals or gamma-globulin from an immune animal. Passive transfer of immunity with immune globulins or gamma-globulins is used in numerous acute situations of infection (diphtheria, tetanus, measles, rabies, etc.), poisoning (insects, reptiles, botulism), and as a prophylactic measure (hypogammaglobulinemia). In these situations, gamma-globulins of human origin are preferable, although specific antibodies raised in other species are effective and used in some cases (poisoning, diphtheria, tetanus, gas gangrene, botulism). While this form of immunization has the advantage of providing immediate protection, heterologous gamma-globulins are effective for only a short duration and often result in pathological complications (serum sickness) and anaphylaxis. Homologous immunoglobulins also carry the risk of transmitting hepatitis and HIV.

Passive transfer of cell-mediated immunity can also be accomplished in certain diseases (cancer, immunodeficiency). However, it is difficult to find histocompatible (matched) donors and there is severe risk of graft versus host disease.

ACTIVE IMMUNITY

This refers to immunity produced by the body following exposure to antigens.

Naturally acquired active immunity. Exposure to various pathogens leads to sub-clinical or clinical infections which result in a protective immune response against these pathogens.

Artificially acquired active immunity. Immunization may be achieved by administering live or dead pathogens or their components. Vaccines used for active immunization consist of live (attenuated) organisms, killed whole organisms, microbial components or secreted toxins (which have been detoxified).

Live vaccines. The first live vaccine was cowpox virus introduced by Edward Jenner as a vaccine for smallpox; however, variolation (innoculation using pus from a patient with a mild case of smallpox) has been in use for over a thousand years.

Live vaccines are used against a number of viral infections (polio (Sabin vaccine), measles, mumps, rubella, chicken pox, hepatitis A, yellow fever, etc.). The only example of live bacterial vaccine is one

against tuberculosis (Mycobacterium bovis: Bacille Calmette-Guerin vaccine: BCG). This is is used in many African, European and Asian countries but not in the United States. Whereas many studies have shown the efficacy of BCG vaccine, a number of studies also cast doubt on its benefits.

Live vaccines normally produce self-limiting non-clinical infections and lead to subsequent immunity, both humoral and cell-mediated, the latter being essential for intracellular pathogens. However, they carry a serious risk of causing overt disease in immunocompromised individuals. Furthermore, since live vaccines are often attenuated (made less pathogenic) by passage in animals or thermal mutation, they can revert to their pathogenic form and cause serious illness. It is for this reason that live polio (Sabin) vaccine, which was used for many years, has been replaced in many countries by the inactivated (Salk) vaccine.

Killed vaccines. Killed (heat, chemical or UV irradiation) viral vaccines include those for polio (Salk vaccine), influenza, rabies, influenza, rabies, etc. Most bacterial vaccines are killed organisms (typhoid, cholera, plague, pertussis, etc.)

Sub-unit vaccines. Some anti-bacterial vaccines utilize purified cell wall components (haemophilus, pertussis, meningococcus, pneumococcus, etc.). Some viral vaccines (hepatitis-B, etc.) consist of purified antigenic proteins manufactured after expression from a gene cloned into a suitable vector (e.g., yeast). When the pathogenic mechanism of an agent involves a toxin, a modified form of the toxin (toxoid, which has lost its toxicity while remaining immunogenic) is used as a vaccine (e.g., diphtheria, tetanus, cholera). These subunit vaccines are designed to reduce the toxicity problems. Each type of vaccine has its own advantages and disadvantages.

Subunit vaccines may consist of proteins or polysaccharides. Since polysaccharides are relatively weak T-independent antigens, and produce only IgM responses without immunologic memory, they are made more immunogenic and T-dependent by conjugation with proteins (e.g., haemophilus, meningococcus, pneumococcus, etc.).

Other novel vaccines. A number of novel approaches to active immunization are in the investigative stage and are used only experimentally. These include anti-idiotype antibodies, DNA vaccines and immunodominant peptides (recognized by the MHC molecules) and may be available in the future.

Anti-idiotype antibodies against polysaccharide antibodies produce long lasting immune responses with immunologic memory.

DNA vaccines (viral peptide genes cloned into vectors) must be injected. They transfect host cells and consequently produce a response similar to that produced against live-attenuated viruses (both cell-mediated and humoral). Several anti-HIV DNA vaccines have been developed but none has so far shown much efficacy.

Immunodominant peptides are simple and easy to prepare and, when incorporated into MHC polymers, can provoke both humoral and cell mediated responses.

Adjuvants. Weaker antigens may be rendered more immunogenic by the addition of other chemicals. Such chemicals are known as adjuvants. There are many biological and chemical substances that have been used in experimental conditions (Table 4. 1). However, only aluminum salts (alum) are approved for human use and it is incorporated in DTP vaccine. Furthermore, pertussis itself has adjuvant effects. Adjuvants used experimentally include mixtures of oil and detergents, with (Freund's complete adjuvant) or without (Freund's incomplete adjuvant) certain bacteria. Bacteria most often used in an adjuvant are Mycobacteria (BCG) and Nocardia. In some instances, sub-cellular fractions of these bacteria can also be used effectively as adjuvants. Newer adjuvant formulations include synthetic polymers and oligonucleotides. Most adjuvants recognize TOLL-like receptors, thus activating mononuclear phagocytes and inducing selective cytokines that can enhance Th1 or Th2 responses, depending on the nature of the adjuvant.

Table 4. 1. Selected adjuvants in clinical or experimental use

Adjuvant type	human use	Experimental only
Salts: aluminum hydroxide, aluminum phosphate-calcium phosphate	 Yes Yes	Slow release of antigen, TLR interaction and cytokine induction
Beryllium hydroxide	No	
Synthetic particles: Liposomes, ISCOMs, polylactates	 No No	Slow release of antigen
Polynucleotides: CpG and others	No*	TLR interaction and cytokine induction
Bacterial products: *B.pertussis*	Yes	TLR interaction and cytokine induction
M. bovis (BCG and others)	No	
Mineral oils	No	Antigen depot
Cytokines: IL-1, IL-2, IL12, IFN-γ, *etc*.	 No*	Activation and differentiation of T- and B cells and APC

*Experimental use in human malignancies

The protective immunity conferred by a vaccine may be life-long (measles, mumps, rubella, small pox, tuberculosis, yellow fever, etc.) or may last as little as a few months (cholera). The primary immunization may be given at the age of 2 to 3 months (diphtheria, pertussis, tetanus, polio), or 13 to 15 months (mumps, measles, rubella). This schedule is revised on a yearly basis or as need by the CDC Advisory Committee on Immunization Practice (AICP).

Prophylactic versus therapeutic immunization. Most vaccines are given prophylactically, i.e. prior to exposure to the pathogen. However, some vaccines can be administered therapeutically, i.e. post exposure (e.g., rabies virus). The effectiveness of this mode of immunization depends on the rate of replication of the pathogen, incubation period and the pathogenic mechanism. For this reason, only a booster shot with tetanus is sufficient if the exposure to the pathogen is within less than 10 years and if the exposure is minimal (wounds are relatively superficial). In a situation where the pathogen has a short incubation period, only a small amount of pathogenic molecules could be fatal (e.g., tetanus and diphtheria); therefore both passive and active post exposure immunization are essential. This is also the case when a bolus of infection is relatively large

Passive prophylactic immunization is also normal in cases of defects in the immune system, such as hypogammaglobulinemias.

Table 4. 2. Approximate rates of adverse event occurring within 48 hours DTP vaccination

Event	Frequency
Local	
redness, swelling, pain	1 in 2-3 doses
Mild/moderate systemic	
fever, drowsiness, fretfulness	1 in 2-3 doses
vomiting, anorexia	1 in 5-15 doses
More serious systemic	
persistent crying, fever	1 in 100-300 doses
collapse, convulsions	1 in 1750 doses
acute encephalopathy	1 in 100,000 doses
permanent neurological deficit	1 in 300,000 doses

Adverse effects of immunization. Active immunization may cause fever, malaise and discomfort. Some vaccine may also cause joint pains or arthritis (rubella), convulsions, that may sometimes be fatal (pertussis), or neurological disorders (influenza). Allergies to eggs may develop as a consequence of viral vaccines produced in eggs (measles, mumps, influenza, yellow fever). Booster shots result in more pronounced inflammatory effects than the primary immunization. The serious side effects have been documented after use of the DTP vaccine (Table 4. 2). Most of these were attributable to the whole pertussis component of the vaccine and have been eliminated by the use of an acellular pertussis preparation.

THEME №5. PRIMARY IMMUNE DEFICIENCIES

SUMMARY

- Humoral immune deficiency refers to diseases resulting from impaired antibody production, because of either a molecular defect intrinsic to B cells or a failure of interactions between B and T cells.
- Antibody deficiency characteristically leads to recurrent, often severe, upper and lower respiratory tract infections with encapsulated bacteria (eg, Streptococcus pneumoniae, Haemophilus influenzae).
- Findings associated with severe primary humoral immune deficiencies include failure to thrive, chronic diarrhea, recurrent fever, nodular lymphoid hyperplasia in the gut, and hepatosplenomegaly.
- Evaluation of suspected primary humoral immune deficiency includes quantitative immunoglobulin levels, specific antibody titers, and enumeration of B cells and B cell subpopulations.

The term primary immunodeficiency disease denotes disorders resulting from the mostly inherited defects of the immune system. Multiple isolated defects and combined disorders have been described, including humoral immune deficiencies, the severe combined immunodeficiencies, and disorders resulting from phagocytic and complement defects.

The symptoms, signs, and molecular pathophysiology of the major humoral immune deficiencies will be reviewed here, as well as pertinent aspects of the laboratory evaluation and differential diagnosis. An overview of the laboratory abnormalities observed in the different primary humoral immune deficiencies is presented in the table. General considerations and descriptions of methods used in the evaluation of immune system function are discussed separately.

Primary immunodeficiencies are inherited defects of the immune system (figure 5. 1). These defects may be in the specific or non-specific immune mechanisms. They are classified on the basis of the site of lesion in the developmental or differentiation pathway of the immune system.

Individuals with immunodeficiencies are susceptible to a variety of infections and the type of infection depends on the nature of immunodeficiency (Table 5. 1).

SPECIFIC IMMUNE SYSTEM

There are variety of immunodeficiencies which result from defects in stem cell differentiation and may involve T-cells, B-cells, and/or immunoglobulins of different classes and subclasses.

A defect in the early hematopoiesis which involves stem cells results in reticular dysgenesis that leads to general immune defects and subsequent susceptibility to infections. This condition is often fatal but very rare. It can be treated successfully by bone marrow transplantation.

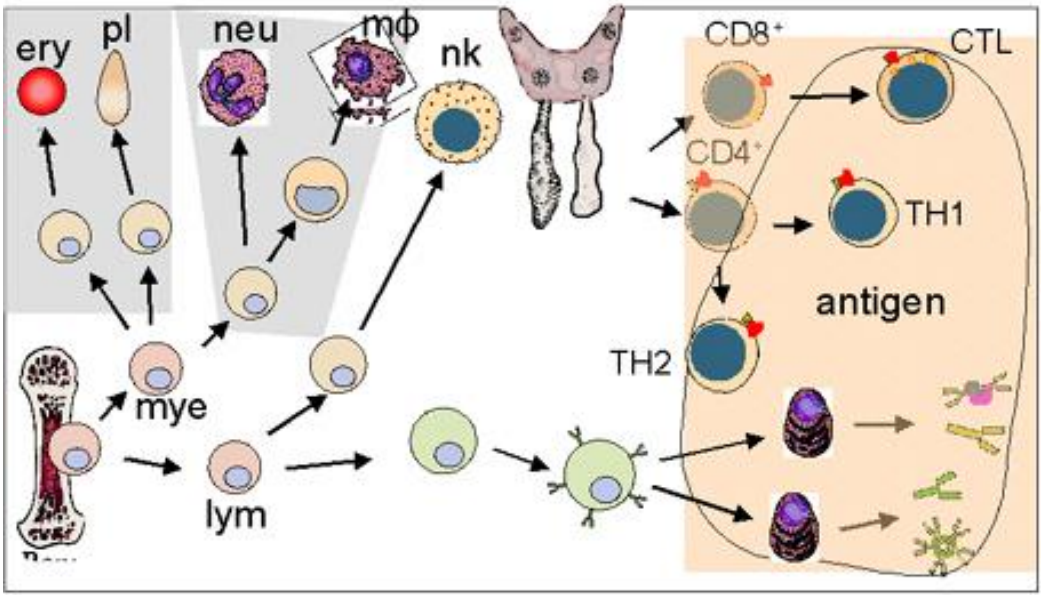

Figure 5. 1. Primary immunodeficiencies are inherited defects of the immune system

Table 5. 1. Characteristic infections of the primary immunodeficiencies

Component	Primary pathogen	Primary site	Clinical example
T-cells	Intracellular, bacteria viruses, protozoa, fungi,	non-specific	SCID, DiGeorge
B-cells	Pneumococcus,Streptococcus, Haemophilus	lung, skin, CNS	IgG, IgM deficiency
	Enteric bacteria and viruses	GI, nasal, eye	IgA deficiency
Phagocytes	Staphylococcus, Klebsiella Pseudomonas	lung, skin, regional lymph node	chronic granulomatous disease (CGD)
Complement	Neisseria, Haemophilus,Pneumococcus,Streptococcus	CNS, lung, skin	C3, Factors I and H, late C components

Lymphoid lineage immunodeficiency

If the lymphoid progenitor cells are defective, then both the T and B cell lineages are affected and result in the severe combined immunodeficiency (SCID). Infants suffer from recurrent infections especially by opportunistic microrganisms (bacterial, viral, mycotic and protozoan infections).

In about 50% of SCID patients, the immunodeficiency is x-linked whereas in the other half the deficiency is autosomal. Both are characterized by an absence of T cell and B cell immunity and absence (or very low numbers) of circulating T and B lymphocytes. Thymic shadows are absent on X-rays.

The x-linked severe SCID is due to a defect in the gamma-chain of IL-2 also shared by IL-4, -7, -11 and 15, all of which are involved in lymphocyte proliferation and/or differentiation. The autosomal SCIDs arise primarily from defects in adenosine deaminase (ADA) or purine nucleoside phosphorylase (PNP) genes which results is accumulation of dATP or dGTP, respectively, and cause toxicity to lymphoid stem cells.

Other genetic defects leading to SCID include those for RAG1, RAG2 and IL-7-alpha. If suspected of SCID, the patient must not receive live vaccine, as it will result in progressing disease.

Diagnosis is based on enumeration of T and B cells and immunoglobulin measurement. Severe combined immunodeficiency can be treated with a bone marrow transplant. Recently, autosomal SCID patients with ADA deficiency have been treated with a retroviral vector transfected with the gene with some success.

SCID INCLUDES SEVERAL DISORDERS

Recombinase activating genes. Patients having both T and B cell deficiency lack recombinase activating genes (RAG1 and 2) that are responsible for the T cell receptor and immunoglobulin gene rearrangements. These patients are athymic and are diagnosed by examining the T cell receptor (TCR) gene rearrangement. Defects in B cells are not observed in early infant life because of passive antibodies obtained from the mother. NK cells are normal in these patients. This is an autosomal recessive trait.

CD3 chain. In some SCID patients, T cells may be present but functionally defective because of deficiency in signaling mediated by the CD3 chain that is associated with the TCR.

Interleukin-2 receptor. Interleukin-2 receptor common gamma chain (IL-2Rγc) may be lacking in patients thereby preventing signaling by IL-2 and other cytokines which act as growth factors. This leads to a defect in the proliferation of T cells, B cells and NK cells. This is an autosomal recessive trait.

Adenosine deaminase. Adenosine deaminase (ADA) is an enzyme responsible for converting adenosine to inosine. ADA deficiency leads to accumulation of adenosine which results in the production of toxic metabolites that interfere with DNA synthesis. The patients have defects in T, B and NK cells.

DISORDERS OF T CELLS

T cell disorders affect both cell-mediated and humoral immunity making the patient susceptible to viral, protozoal and fungal infections. Viral infections such as those by cytomegalovirus and attenuated measles in the vaccine can be fatal in these patients.

DiGeorge's Syndrome (Deletion 22 Syndrome). This the most clearly defined T-cell immunodeficiency and is also known as congenital thymic aplasia/hypoplasia, or immunodeficiency with hypoparathyroidism. The syndrome is associated with hypoparathyroidism, congenital heart disease, low set notched ears and fish shaped mouth. These defects results from abnormal development of the fetus (3rd and 4th pharyngeal pouch) during the 6th to 10th week of gestation when parathyroid, thymus, lips, ears and aortic areh are being formed. No genetic predisposition is clear and not all DiGeorge syndrome babies have thymic aplasia. A thymic graft taken from an early fetus (13 - 14 weeks of gestation) can be used for treatment. Older grafts may result in GVH reaction. In severely immunodeficient DiGeorge patients, live vaccines may cause progressive infections.

DiGeorge syndrome is autosomal dominant and is caused by a deletion in chromosome 22. The deletions are of variable size but size does not correlate with severity of disease. In about 6% of cases, the chromosome 22 microdeletion is inherited but most cases result from de novo deletion which may be caused by environmental factors. Patients may be treated with a thymic graft.

T CELL DEFICIENCIES WITH VARIABLE DEGREES OF B CELL DEFICIENCY

Ataxia-telangiectasia. Ataxia-telangiectasia is a deficiency of T cells associated with a lack of coordination of movement (ataxis) and dilation of small blood vessels of the facial area (telangiectasis). T-cells and their functions are reduced to various degrees. B cell numbers and IgM concentrations are normal to low. IgG is often reduced and IgA is considerably reduced (in 70% of the cases). There is a high incidence of malignancy, particularly leukemias, in these patients. The defects arise from a breakage in chromosome 14 at the site of TCR and immuinoglobulin heavy chain genes.

Wiskott-Aldrich syndrome. Wiskott-Aldrich syndrome syndrome is associated with normal T cell numbers with reduced functions, which get progressively worse. IgM concentrations are reduced but IgG levels are normal. Both IgA and IgE levels are elevated. Boys with this syndrome develop severe eczema, petechia (due to platelet defect and thrombocytopenia). They respond poorly to polysaccharide antigens and are prone to pyogenic infection. Wiskott-Aldrich syndrome is an X-linked disorder (figure 4) due to defect in a cytoskeletal glycoprotein, CD43.

MHC deficiency (Bare leukocyte syndrome). A number of cases of immunodeficiency have been described in which there is a defect in the MHC class II transactivator (CIITA) protein gene, which results in a lack of class II MHC molecules on their APC. Since the positive selection of CD4 cells in the thymus depends on the presence of these MHC molecules, these patients have fewer CD4 cells and are infection

prone. There are also individuals who have a defect in their transport associated protein (TAP) gene and hence do not express the class I MHC molecules and consequently are deficient in CD8+ T cells.

DISORDERS OF B LYMPHOCYTES.

There are a number of diseases in which T cell numbers and functions are normal: B cell numbers may be low or normal but immunoglobulin levels are low.

Humoral immune deficiency refers to diseases resulting from impaired antibody production, because of either a molecular defect intrinsic to B cells or a failure of interactions between B and T cells Cellular immunity is largely intact, in contrast to diseases classified as combined immunodeficiencies, despite underlying T cell defects in some of these diseases.

Antibody deficiency characteristically leads to recurrent, often severe, upper and lower respiratory tract infections with encapsulated bacteria (eg, Streptococcus pneumoniae, Haemophilus influenzae) Children commonly present with recurrent otitis media, sinusitis, and pneumonia. The same is true of adults, although otitis media is less common. Viral infections of the respiratory tract also occur with greater frequency and severity in these patients Additional infectious diseases may be associated with particular syndromes.

Common associated findings in children include poor growth and failure to thrive, recurrent fevers, and poor school attendance and performance Chronic diarrhea is seen in both children and adults Autoimmune disease is occasionally the presenting feature in adults. Primary humoral immune deficiency should also be considered in patients with nodular lymphoid hyperplasia in the gut or unexplained hepatosplenomegaly.

Patients with suspected antibody deficiency should have measurement of total serum IgG, IgA, and IgM. The importance of IgD, IgE, and IgG subclass levels is controversial.

Specific antibody titers to protein (eg, tetanus, diphtheria) and polysaccharide (eg, pneumococcal polysaccharides, H. influenzae type B capsular polysaccharide) antigens should also be measured. If specific antibody levels are low, booster immunization should be administered and titers measured again four weeks later. Isohemagglutinins may be measured in infants who have not completed a primary series of immunizations, since such antibodies are usually detectable by six months of age.

Transient hypogammaglobulinemia. Children, at birth, have IgG levels comparable to that of the mother. Because the half life of IgG is about 30 days, its level gradually declines, but by three months of age normal infants begin to synthesize their own IgG. In some infants, however, IgG synthesis may not begin until they are 2 to 3 years old. This delay has been attributed to poor T cell help. This results in a transient deficiency of IgG which can be treated with gamma-globulin.

Common variable hypogammaglobulinemia (Late onset hypogammaglobulinemia). These individuals have deficiencies of IgG and IgA in the 2nd or 3rd decade of their life because B cells fail to differentiate into plasma cells. These patients are susceptible to a variety of pyogenic bacteria and intestinal protozoa. They should be treated with specially prepared gamma-globulin for intravenous use.

IgA deficiency. IgA deficiency is the commonest of all immunodeficiencies (1/700 of all Caucasians) and results from a defect in class switching. About 20% of individuals with IgA deficiency also have low IgG. IgA-deficient patients are very susceptible to gastrointestinal, eye and nasopharyngeal infections. Patients with IgA deficiency have a high incidence of autoimmune diseases (particularly immune complex type) and lymphoid malignancies. Anti-IgA antibodies (IgG) are detected in 30 to 40 percent of patients who should not be treated with γ-globulins. Laboratory diagnosis is based on IgA measurement. IgA deficiency — IgA deficiency is a relatively common immunologic variant, which is asymptomatic in the majority of affected individuals. In a small minority, however, it can be associated with autoimmune, gastrointestinal, and atopic disorders.

Selective antibody deficiency with normal immunoglobulins — Selective antibody deficiency with normal immunoglobulins (SADNI) (also referred to as polysaccharide nonresponsiveness,

polysaccharide nonresponse, impaired polysaccharide responsiveness, or specific antibody deficiency) is a condition that may exist alone, or as part of a more global immunodeficiency. It can be defined as unresponsiveness to polysaccharide antigens. Symptomatic patients with this isolated defect may have recurrent sinopulmonary infections.

Selective IgG deficiency. — There are four IgG subclasses: IgG1, IgG2, IgG3, and IgG4. IgG subclass deficiency is defined as the relative lack of one or more IgG subclasses, with a normal or near normal concentration of total serum IgG in a patient with recurrent severe infections. These disorders are discussed in more detail separately. Deficiencies of different IgG subclasses have been found. These patients are susceptible to pyogenic infections.

Selective IgM deficiency — Selective deficiency of immunoglobulin M (sIgM-D) is a rare immune disorder that has been reported in association with bacteremia and other serious infections. Patients with selective IgM deficiency may be asymptomatic, have repeated infections, and/or present with associated conditions, such as autoimmune, malignant, and hematologic disorders or atopic diseases.

Selective IgE deficiency — Selective IgE deficiency is defined as a level of IgE <2.5 IU/mL in a patient whose other immunoglobulin levels and IgG subclass levels are normal. It is a laboratory finding that does not necessarily equate to a clinical disorder.

Hyperimmunoglobulin-D syndrome — The hyperimmunoglobulin-D syndrome (HIDS) is a rare genetic disorder characterized by recurrent febrile episodes typically associated with lymphadenopathy, abdominal pain, and an elevated serum polyclonal IgD level. HIDS is one of several disorders involving recurrent fevers that are not due to infection. These conditions are considered autoinflammatory diseases, rather than immunodeficiencies.

X-linked Hyper-IgM immunodeficiency. Hyper-IgM syndrome (HIGM), also called "immune deficiency with normal or elevated IgM", is a somewhat obsolete term denoting deficiency of IgG, IgA, and IgE, with normal or elevated serum concentrations of IgM. Several gene defects have since been identified within this group of disorders, and it is preferable to refer to the specific defect to avoid confusion.

Three disorders have been reclassified as combined immunodeficiencies because of associated abnormalities of cellular immunity:

The most common defect is X-linked hyper IgM syndrome due to deficiency of CD40 ligand (CD40L, also called CD154, or TNFSF5 for tumor necrosis factor superfamily member 5). An autosomal recessive form due to deficiency of CD40 (TNFRSF5 or tumor necrosis family receptor superfamily member 5) is also recognized.

Another disorder, termed NEMO deficiency, is discussed separately.

Two additional rare forms of hyper-IgM syndrome, with autosomal recessive inheritance and normal T cell function, are caused by defects in enzymes required for the process of immunoglobulin class-switching. Specifically, mutations in nucleic acid modifying enzymes called activation-induced cytidine deaminase (AICDA or AID, MIM 605258) and uracil nucleoside glycosylase (UNG, MIM 608106) have been demonstrated. B cells cannot change isotype production from IgM to IgG, IgA, or IgE during antigen-dependent development and thus only produce appreciable quantities of IgM.

No specific defects have been identified in an additional group of patients with HIGM with residual IgG production (HIGM type IV).

Clinical manifestations — Patients with AICDA and UNG deficiencies generally present with the recurrent and severe sinopulmonary infections with encapsulated bacteria that are characteristic of antibody deficiencies Gastrointestinal infections (including Giardiasis), bacterial meningitis, viral encephalitis, and severe hepatitis B infections have also been observed.

Lymphoid hyperplasia is seen in about two-thirds of patients. This may involve peripheral nodes, as well as mesenteric nodes, tonsils, liver, and spleen.

A variety of autoimmune pathologies have also been reported in these patients, including diabetes mellitus, autoimmune hepatitis, rheumatoid arthritis, inflammatory bowel disease, and uveitis.

Laboratory findings — Among those with AICDA and UNG deficiencies, IgG levels are generally <200 mg/dL, while IgA is <20 mg/dL, and IgM ranges from 100 to 3700 mg/dL. IgG specific antibody responses are nonexistent. Some patients have IgM isohemagglutinins. Numbers of T and B cells in the circulation are normal, and T cell function is intact Tests for both AICDA and UNG deficiency are commercially available.

Treatment — Patients with AICDA and UNG deficiencies generally exhibit excellent responses to immune globulin replacement therapy with reduction in bacterial infections. Lymphoid hyperplasia is also reduced, but does not disappear in most. Treatment of autoimmune diseases requires antiinflammatory and immunosuppressive medications.

Spontaneous resolution by the age of approximately six years was reported in some patients with HIGM type IV who do not have identifiable defects or deficiency in CD40L, CD40, NEMO, UNG or AICDA Patients with this type of HIGM should be reevaluated periodically to see if immune function has normalized.

NON-SPECIFIC IMMUNE SYSTEM - DEFECTS IN THE MYELOID LINEAGE

Primary immunodeficiencies of the non-specific immune system include defects in phagocytic and NK cells and the complement system.

Congenital Agranulomatosis. Patients have a decrease in the neutrophil count. This is due to a defect in the myeloid progenitor cell differentiation into neutrophils. These patients are treated with granulocyte-macrophage colony stimulating factor (GM-CSF) or G-CSF.

Defects of the phagocytic system. Defects of phagocytic cells (numbers and/or functions) can lead to increased susceptibility to a variety of infections.

Cyclic neutropenia. This is marked by low numbers of circulating neutrophil approximately every three weeks. The neutropenia lasts about a week during which the patients are susceptible to infection. The defect appears to be due to poor regulation of neutrophil production.

Chronic granulomatous disease (CGD). CGD is characterized by marked lymphadenopathy, hepato- splenomegaly and chronic draining lymph nodes. Leukocytes have poor intracellular killing (figure 5) and low respiratory burst. In majority of these patients, the deficiency is due to a defect in NADPH oxidase (cytochrome b558: gp91phox, or rarely gp22phox) or other cofactor proteins (gp47phox, gp67phox) that participate in phagocytic respiratory burst. These patients can be diagnosed on the basis of poor Nitroblue tetrazolium (NBT) reduction which is a measure of respiratory burst. Interferon-gamma therapy has been successful.

Leukocyte Adhesion Deficiency. In this disease, T cells and macrophages lack the complement receptor CR3 due to a defect in CD11 or CD18 peptides and consequently they cannot respond to C3b opsonin. Alternatively there may a defect in integrin molecules, LFA-1 or mac-1 arising from defective CD11a or CD11b peptides, respectively. These molecules are involved in diapedesis and hence defective neutrophils cannot respond effectively to chemotactic signals. Treatment is with bone marrow (devoid of T cells and MHC-matched) transplantation or gene therapy.

Chediak-Higashi syndrome. Chediak-Higashi syndrome is marked by reduced (slower rate) intracellular killing and chemotactic movement accompanied by inability of phagosome and lysosome fusion and proteinase deficiency. Giant lysosomes (intracellular granules) are often seen. The respiratory burst is normal. Accompanying NK cell defects and platelet and neurological disorders are noted.

DISORDERS OF COMPLEMENT SYSTEM

Complement abnormalities also lead to increased susceptibility to infections. There are genetic deficiencies of various components of complement system, the most serious of which is the C3 deficiency which may arise from low C3 synthesis or deficiency in factor I or factor H.

THEME №6. SECONDARY IMMUNE DEFICIENCY

Immune system function is altered by many conditions which primarily impair function of other organ systems (table 6. 1). As with primary immune deficiency, secondary immune dysfunction leads to an increased incidence of infection and malignancy, and the occurrence of autoimmune disease.

Table 6. 1 Conditions associated with secondary immune deficiency

Immunosuppressive therapy	**Microbial infection**
• Cytotoxic chemotherapy for malignancy • Treatment of autoimmune disease • Bone marrow ablation prior to transplantation • Treatment or prophylaxis of graft vs. host disease following bone marrow transplantation • Treatment of rejection following solid organ transplantation	• Viral infection • HIV, AIDS • Measles • Herpes viruses • Bacterial infection (superantigens) • Mycobacterial infection • Parasitic infestation
Malignancy	**Disorders of biochemical homeostasis**
• Hodgkin's disease • Chronic lymphocytic leukemia • Multiple myeloma • Solid tumors	• Diabetes mellitus • Renal insufficiency/dialysis • Hepatic insufficiency/cirrhosis • Malnutrition
Autoimmune disease	**Environmental exposure**
• Systemic lupus erythematosus • Rheumatoid arthritis	• Radiation • Ionizing • Ultraviolet
Other	**Trauma, Burns, Toxic chemicals**
• Pregnancy • Stress • Asplenia/hyposplenism	

The mechanisms and sequelae of the immune dysfunction, occurring as the result of biochemical abnormalities, environmental exposures, miscellaneous disorders, and infections other than HIV will be reviewed here. Secondary immune deficiencies resulting from immunosuppressive agents and malignancy are discussed separately.

Infection with the human immunodeficiency virus (HIV) and the acquired immunodeficiency syndrome (AIDS) constitute an entire discipline by themselves, and are discussed separately in the appropriate topic reviews.

DISORDERS OF BIOCHEMICAL HOMEOSTASIS

Disease processes that lead to chronic imbalances in hormones, nutrients, and toxic metabolic waste products in body fluids may have profound effects on the function of one or more components of the immune system. There are a great many diagnostic entities that may be grouped under this broad heading.

It may be that many have as yet unknown effects on immune function. A few disorders where clinically significant immune dysfunction is regularly encountered are presented in this section.

Diabetes mellitus — Neutrophil dysfunction underlies much of the predisposition to fungal infections found in patients with diabetes. The decreased neutrophil function is directly related to the level of hyperglycemia. In addition, poor peripheral circulation leads to skin ulceration, and diminished delivery of neutrophils to sites of microbial entry. Some characteristic infectious complications of diabetes include disseminated candidiasis, rhinopulmonary zygomycosis (mucormycosis), and malignant otitis due to P. aeruginosa. One epidemiologic study indicated that most or all of the increased risk of infection-related mortality in patients with diabetes is due to associated cardiovascularеompromise.

Dialysis and uremia — Patients receiving hemodialysis display reduced T cell function in vitro and in vivo (cutaneous anergy), diminished antibody production, and compromised neutrophil and dendritic function. Compromised neutrophil function may be due in part to the use of bioincompatible dialysis membranes, resulting in impaired adherence and attenuated responses to phagocytic stimuli. Low expression and/or function of IgG Fc receptors have also been noted. Some of these immune defects may be partly explained by the presence of high endogenous glucocorticoid levels. Patients with end stage renal disease, regardless of dialysis treatment, have very high blood levels of soluble interleukin-2 receptor and this may be responsible for reducing the bioavailability of and diminishing in vitro T cell responses to IL-2.

Patients undergoing chronic peritoneal dialysis do not display systemic immune defects. However, peritoneal neutrophil function is depressed as a result of the removal of opsonic factors (immunoglobulin and complement) with the dialysate, as well as directly suppressive effects of the dialysate itself. These features, together with the presence of an indwelling foreign body, explain the susceptibility to bacterial peritonitis observed in these patients.

Cirrhosis — Reduced hepatic metabolism in cirrhosis leads to high levels of endogenous glucocorticoids, which may partly explain the immune dysfunction associated with liver disease. In addition, shunting of portal blood reduces the ability of hepatic Kupffer cells to clear opsonized particles, and hypocomplementemia reduces serum opsonic activity. The most common infectious complications of severe cirrhosis are sepsis, and bacterial peritonitis.

Malnutrition — Most studies on nutritionally-determined immune suppression have focused on protein-energy malnutrition. This is associated with a spectrum of immune defects including cutaneous anergy, diminished T cell mitogen responses, and decreased phagocytic cell function. Additional abnormalities include the following:

- The number of circulating T cells declines, while the percentage of natural killer cells rises.
- Serum immunoglobulin is normal or increased; however, specific antibody responses are impaired.
- Primary and secondary lymphoid organs are relatively depleted of cells, and lymphoid follicles are sparse.

An acute lowering of food intake may also severely affect immune function. One study, for example, found depression of circulating lymphocytes and interleukin-2 production following mitogen stimulation after a fast of only seven days.

Malnutrition predisposes to a greater incidence of clinically apparent infection, and increased morbidity and mortality due to infection with the pathogens prevalent in a given geographic area. It is estimated that worldwide, for example, malnutrition leads to 10- and 30-fold increased mortality from pneumonia and gastroenteritis, respectively. In Latin America, malnutrition is a contributing factor in approximately 60 percent of the deaths due to infection. In a study conducted in rural Bangladesh, the severity of malnutrition was linked to the rate of symptomatic upper respiratory infection. Reduced blood

levels of the hormone leptin may be an important pathway in the immune, endocrine and neurological dysregulation associated with starvation.

A similar spectrum of defects and increased susceptibility to infection has also been linked to restricted nutritional deficiencies of zinc, iron, folate, pyridoxine, and vitamin A. Immune function returns to normal when proper nutritional balance is restored.

DISORDERS OF PROTEIN LOSS

Certain disorders, such as nephrotic syndrome, protein losing enteropathies, severe dermatitis, peritoneal dialysis, and rare pulmonary diseases, can result in hypogammaglobulinemia due to loss of protein via the kidneys, intestinal tract, lymphatic system, or skin.

Hypogammaglobulinemia from protein loss may present as low IgG and IgA, sometimes with near normal IgM. Often antibody levels are present in low titer and as a result, the patient may not have increased susceptibility to infection. Accelerated loss of IgG globulin can be documented by giving a large bolus of immune globulin (IVIG, 1 to 2 grams/kg) and then assessing daily IgG levels. A half life, after equilibration, of less than 15 days suggests protein loss.

Nephrotic syndrome — Patients with nephrotic syndrome can develop hypogammaglobulinemia due to protein loss, as well as depressed cellular immunity due to loss of vitamin D and other serum factors. Treatment with immunosuppressive drugs, such as glucocorticoids, further increases the risk of infection.

Hypogammaglobulinemia may be severe with total IgG less than 200 mg/dL. Infectious complications of nephrotic syndrome include recurrent respiratory tract infections, urinary tract infections, peritonitis, and sepsis, particularly with encapsulated bacteria such as Streptococcus pneumonia. Varicella infections are also problematic in patients requiring immunosuppression. Prevention includes vaccination and careful attention to early symptoms, as discussed separately.

Peritoneal dialysis — Many patients undergoing regular peritoneal dialysis for chronic renal disease develop hypogammaglobulinemia; this may contribute to their defective peritoneal defenses.

Protein losing enteropathies — A variety of gastrointestinal disorders can result in protein loss and hypogammaglobulinemia. More common diseases include celiac disease, inflammatory bowel disease, and intestinal lymphangiectasia. Protein loss should be demonstrable by measurement of the alpha-1 antitrypsin clearance in the stool. Alpha-l antitrypsin has a moderately higher molecular weight than albumin (50,000) and, because it is resistant to proteolysis, is not degraded in the intestinal lumen. Thus, it passes intact into the stool when there is mucosal inflammation.

Intestinal lymphangiectasia — Intestinal lymphangiectasia is abnormal dilatation of intestinal mucosal lymphatic channels leading to loss of lymph with immunoglobulins and lymphocytes into the gut. The disorder may be congenital, or may arise secondarily to processes which obstruct lymph drainage of the gut or raise central venous pressure. Congenital forms may also be associated with pulmonary chylothorax and lymphedema. It may occur as a result of surgery for congenital heart disease, particularly after the Fontan procedure.

Hypogammaglobulinemia and lymphopenia are variable, and some patients have an increased rate of infections, including opportunistic infections. Naive CD4 and CD8 T cells are lost preferentially to memory T cells and NK cells, which are retained. Mitogen proliferative responses are preserved. Similar alterations are observed in patients with chylothorax.

Patients with recurrent infections and low serum IgG may benefit from gamma globulin infusions; however, relatively large doses may be required due to ongoing intestinal loss. Replacement therapy in this setting remains controversial.

Other disorders — Other disorders that can result in hypogammaglobulinemia due to protein loss include severe dermatitis and plastic bronchitis with chylothorax.

TRAUMA

Trauma is associated with subsequent defects in host defense that are generally proportional to the extent of tissue injury. The mechanism initiating the cascade of immune effects is thought to be the massive release of inflammatory cytokines (interleukin-1, tumor necrosis factor) due to widespread activation of monocytes and macrophages by the products of cellular necrosis.

Burns — Burn trauma tends to result in a relatively greater immune suppression than mechanical trauma, when the extent of injury is similar. The reason for this is not known. In addition to depression of specific immune activation and effector mechanisms, burns also disrupt a relatively large area of nonspecific defense (the skin). This also greatly increases the risk of infection by providing microbes ready access to the interior of the body.

ENVIRONMENTAL EXPOSURES

Environmental exposures that can result in immune dysfunction include ionizing and ultraviolet radiation, and toxic chemicals.

Ionizing radiation — Ionizing radiation (X-rays, gamma rays) damages DNA by causing single and double-stranded breaks, as well as chemical changes in nucleotide base structure. This leads to impaired cell division, as well as to somatic mutations which may be expressed.

Impaired cell division is the main mechanism of impairment of immune system function, and operates in a manner entirely analogous to what has been described for chemotherapeutic immune suppressive agents. In addition, radiation may induce apoptosis (programmed cell death) in susceptible lymphocyte populations. Somatic mutations may impair the function of cellular proteins that regulate cell division (eg, the p53 tumor suppressor gene), and lead to malignant cell growth.

Radiation induces a rapid (hours) dose-dependent decline in peripheral blood lymphocyte counts. B cells are more sensitive to radiation than T cells, as reflected in the depletion of germinal centers and other B cell rich areas of irradiated lymph nodes and spleens. Lymphocyte homing and recirculation are also affected, such that lymphocytes do not properly traffic between different lymphoid organs and regions of the body. In general, T cell numbers recover more rapidly following irradiation in comparison to B cells.

Thymic cortical cells are undergoing rapid cell division, and are more radiosensitive than medullary thymocytes. Other thymic cell populations (epithelial cells) are relatively radioresistant. Several decades ago, when an "enlarged" thymus was considered a risk factor for infant mortality, the thymus was irradiated to reduce its size. This frequently resulted in long-lasting depression in blood T cell counts, as well as reduction in the in vitro response to mitogens. In vivo cutaneous delayed type hypersensitivity (DTH) responses were relatively preserved. However, these children showed several delayed effects including a higher rate of thymoma, as well as allergic and autoimmune diseases such as asthma, vasculitides, sareoidosis, inflammatory bowel disease, and thyroiditis. The specific tolerance mechanisms which are affected by radiation leading to autoimmune disease have not been defined.

Primary antibody responses are most often diminished by whole body irradiation, both due to suppression of proliferation and/or induction of apoptosis in B and T cells. As after thymic irradiation, DTH responses and in vitro cellular allocytotoxicity are relatively intact following whole body irradiation. In addition, most functions of mature, long-lived phagocytic cells, such as macrophages, appear to be relatively radiation-resistant.

Even regional radiation therapy applied for the treatment of malignancy can have systemic immunologic effects. Radiation treatment of lung cancer may lead to diminished T cell numbers and reduced mitogen proliferative response in vitro.

The increased susceptibility to infection that results from high doses of whole body irradiation arises not only from general bone marrow and lymphocyte suppression, but also from damage to local defensive

barriers. The gastrointestinal tract, and to a slightly lesser extent the skin, are both organs which always sustain a high rate of cell division to replace cell loss. Irradiation interferes with cell replacement and leads to breakdown of these defensive barriers. Fatal infections may be caused not only by common pathogens, but also by normal commensal flora.

Measurable immunologic effects of intense radiation exposure may be long-lived. Japanese studies of survivors of atomic bomb explosions have shown the persistence over 60 years of reduced proportions of helper T cells, diminished in vitro mitogen responses and poor IL-2 production, and reduced serum levels of inflammatory cytokines.

Ultraviolet radiation — Ultraviolet B (UVB) radiation via sun exposure is the major determinant of risk for skin cancer. This occurs through both direct mutagenesis and disruption of the cell cycle in skin epithelial cells, and from suppression of skin immune function. Chronic UV exposure leads to diminished function of all skin resident immune cells including lymphocytes, mast cells, and mononuclear derived cells including macrophages and dendritic cells. Immune suppressive alterations include an increased production of the antiinflammatory cytokine interleukin-10 and an increase in CD25+ regulatory T cells (Treg). These skin Treg cells have been shown to exert direct effects on skin tumorigenesis in UV-exposed mice. Treg cells can be induced in the skin of neonates exposed to UV and may persist for many years, possibly even into adulthood. The implications for long-term health are unknown.

Toxic chemicals — Numerous environmental chemicals have been incriminated in causing harm to the immune system, giving rise to the discipline of immunotoxicology. Many of these reports are anecdotal, and clinical studies frequently suffer from difficulties in the definition of insults to the immune system, small numbers, inadequacy or lack of appropriate controls, insufficient correlation between clinical problems and laboratory observations, quantitation of exposure to the substance in question, and lack of reproducibility of findings.

Nevertheless, accumulated experience supports the importance of environmental chemical exposure for immune system dysfunction. The table lists some of the "xenobiotics" which have been found or suggested to cause immune defects in animals and humans, and associated toxicities. In no case has a specific molecular pathophysiology been described. Many of these compounds are variably bone marrow suppressive, and have also been linked to abnormalities of T cell function in vivo (thymic atrophy, circulating lymphocyte subsets, DTH) and in vitro (mitogen, antigen, and mixed lymphocyte responses, cytotoxicity). Some compounds have also been found to cause polyclonal B cell activation and have been associated with autoimmune phenomena. In some cases, exposure has been found to cause an increased incidence of infection, predominantly of the respiratory tract. Several compounds are also implicated in an increased cancer risk, either through direct mutagenic potential, decreased immune tumor surveillance, or both.

Developmental immunotoxicology has emerged as a subdiscipline. Since the immune system changes significantly from the newborn period through adulthood, the immunologic insults (immune suppression, predisposition to allergy or autoimmunity) resulting from some environmental toxic exposures have different effects depending on age (including gestational age). In general, immunologic (and other) toxic effects are more pronounced in younger developing individuals compared to adults.

ALLOGENEIC BLOOD TRANSFUSION

Blood transfusion from major-histocompatibility-unrelated donors increases the rate of postoperative infection by 30 percent or more. As an example, in a retrospective study of almost 10,000 consecutive hip fracture patients undergoing surgical repair, allogeneic blood transfusion was associated with a significant increase in the risk of serious postoperative bacterial infection (5.2 versus 3.7 percent with no transfusion). There was a significant dose-response relationship between the adjusted hazard

ratios for these two complications and the number of units of allogeneic blood transfused. The mechanism of susceptibility is unknown, but the effect is not seen with leukocyte-depleted blood in animal studies.

One estimate of excess mortality due to infection resulting from blood transfusion-induced immune suppression is 125 deaths/million units. In animal models, blood transfusion also leads to accelerated tumor growth and increased mortality. This may be important in the occurrence and recurrence of malignancy in humans. Blood transfusion increases mortality by 9 percent in patients with colorectal cancer.

NORMAL LIFE STAGES AND EVENTS

Immune function may be impaired by normal life stages and events, such as aging, pregnancy, and extreme stress.

Aging — Immune dysfunction associated with aging is reviewed separately.

Pregnancy — Pregnant women have a higher incidence of numerous infectious diseases dependent upon cellular immunity for their control. These include hepatitis A and B, influenza, herpesviruses, chlamydia, listeria, Campylobacter, tuberculosis, and several fungal, protozoan, and helminthic infections.

Depressed cellular immunity during pregnancy is assumed to have a "survival benefit" by reducing the likelihood of maternal "rejection" of the fetus which contains potent alloantigenic stimuli derived from the father. Multiple etiologic factors have been implicated:

- Progesterone may be a major immunosuppressive factor in pregnancy. It has been shown to inhibit lymphocyte proliferation in vitro.
- A pregnancy-specific serum factor called uromodulin has been shown to inhibit B cell activity, although antibody responses are generally preserved during pregnancy.
- Depressed T cell responses to mitogens have been observed only in the presence of autologous serum, suggesting the importance of circulating suppressive factors.

Stress — Major life stresses such as bereavement, as well as less catastrophic stresses such as examinations in medical school, have been associated with increased rates of respiratory tract infection, reactivation of herpesvirus infections, and increased incidence of cancer. Similar findings occur in humans and animals during and after space flight. While the space environment may play a role, this is thought to be most likely the result of a relatively extreme occupational psychological stress, with possible implications for more down-to-earth highly stressful occupations. Diminished cellular immune function has also been described in those suffering from post-traumatic stress disorder.

Laboratory studies have consistently shown reduced natural killer cell activity and depressed lymphocyte mitogen responses in stressed individuals. The discipline of psychoneuroimmunology is devoted to the study of these phenomena, although well-defined mechanisms of neural regulation of immunity are yet to be described. Increased production of corticotropin-releasing factor and sympathetic autonomic activity has been suggested to play a role.

It is unlikely that emotional stress alone, however severe, will commonly cause an increased incidence or severity of infection sufficient to prompt investigation of immune function. The degree to which chronic stress contributes to the public health burden of infectious disease and malignancy remains a subject of debate.

INFECTIONS

Many human pathogens have evolved sophisticated means for surviving attack by the immune systems of their hosts. In most cases, these mechanisms selectively affect host response to the invader and are not generally immunosuppressive, with the important exception of the profound immune suppression resulting from HIV infection.

Instances in which microbial infection leads to less profound generalized immune suppression will be discussed here. Laboratory studies of immune function are not routinely conducted in patients with these infections.

Viral infections

Immunologic abnormalities in the AIDS

All acquired immunodeficiencies have been outdone by AIDS that is caused by Human Immunodeficiency Virus (HIV)-1. This virus was first discovered in 1981 and the patients exhibited fungal infections with opportunistic organisms such as Pneumocystis carinii and in other cases, with a skin tumor known as Kaposi's sareoma. There are two major types of HIV: HIV-1 and 2, the former being the strain frequently found in North America. HIV is spread through sexual intercourse, infected blood and body fluids as well as from mother to offspring. HIV is a retrovirus with RNA that is reverse transcribed to DNA by reverse transciptase (RT) following entry into the cell. The DNA is integrated into the cell genome as a provirus that is replicated along with the cell. HIV-1 does not replicate in most other animals but infects chimpanzees although it does not induce AIDS in them. Severe combined immunodeficient mice (SCID) reconstituted with human lymphocytes can be infected with HIV-1. The HIV-1 virion consists of a viral envelope made up of the outer lipid bilayer of the host cell in which are embedded glycoproteins composed of the transmembrane gp41 along with the associated gp120. The gp120 binds the CD4 expressed on host cells. Within the viral envelope is the viral core or nucleocapsid consisting of a layer of matrix protein composed of p17 and an inner capsid made up of p24. The viral genome consists of two single stranded RNA molecules associated with two RT molecules as well as other enzymes including a protease and an integrase.

Replication cycle and targets of therapy. The virus attaches to the CD4 molecule on Th cells, monocytes and dendritic cells through the gp120 of HIV. For HIV infection, a co-receptor is required. The co-receptor is a chemokine receptor such as CXCR4 or CCR5. CCR5, expressed predominantly on macrophages, and CXCR4 on CD4+ T cells serve as coreceptors for HIV infection. After the fusion of HIV envelope and the host membrane, the nucleocapsid enters the cell. The RT synthesizes viral DNA which is transported to the nucleus where it integrates with the cell DNA in the form of a provirus. The provirus can remain latent until the cell is activated when the provirus also undergoes transcription. Virions, consisting of the transcribed viral RNA and proteins, are produced. These bud out of the host cell membrane from where they acquire the envelope. Thus, therapeutic agents have been developed that target viral entry and fusion, as well as serve as RT, protease and integrase inhibitors. Highly active anti-retroviral therapy is a cocktail of 3 or more such agents.

Immunological Changes. The virus replicates rapidly and within about two weeks the patient may develop fever. The viral load in the blood increases significantly and peaks in two months, after which there is a sudden decline because of the latent virus found in germinal centers of the lymph nodes. CTL develop very early whereas antibodies can be detected between 3 - 8 weeks. The CTL killing of Th cells around 4 - 8 weeks leads to a decrease in CD4+ T cells. When the CD4+ T cell count decreases below 200 per cubic mm, full blown AIDS develops.

Immunotherapy

There are several barriers to development of an effective HIV vaccine.

- Attenuated vaccine may induce the disease
- CD4+ T cells may be destroyed by the vaccine
- Antigenic variation of HIV
- Low immunogenicity of the virus by downregulation of MHC molecules
- Lack of animal models
- Lack of in vitro tests

The following reagents have been considered in developing vaccines

- Immunization with deletion mutants to reduce pathogenicity
- Vaccination with recombinant proteins
- Gene encoding proteins introduced into virus vectors may be used for vaccination
- Chemokines that compete for the co-receptors
- IL-2 to boost the Th cells.

Measles — Aside from HIV, measles (morbillivirus) is the only viral agent implicated in significant global immune suppression, leading to severe, and sometimes fatal, superinfection. Secondary immunosuppression due to measles virus infection is particularly important in the developing world, and malnutrition is an important independent risk factor for severe immune compromise, superinfection, and death from measles infection. In one retrospective study of measles fatalities in South Africa, 85 percent of deaths were mainly attributed to viral, bacterial, or fungal lung super- or co-infections. The most frequent infectious complications of measles are pneumonia, gastroenteritis, otitis media, gingivostomatitis, and laryngotracheobronchitis. Pathogens included common viral agents such as herpes simplex, cytomegalovirus, parainfluenza, adenovirus, coxsackie, and respiratory syncytial virus. Bacteria included community-acquired organisms such as Staphylococcus aureus and Streptococcus pneumoniae, as well as nosocomial pathogens such as Klebsiella, Pseudomonas, and Acinetobacter. Mycobacterium tuberculosis and Candida albicans were also found.

Immune alterations induced by measles include T cell lymphopenia with depletion of T-dependent areas of lymph nodes and spleen, cutaneous anergy, diminished in vitro T cell proliferation with mitogens or alloantigens, and diminished antibody production. These effects are caused by direct infection of T cells by measles virus and by infection of dendritic cells, impairing their important antigen presenting/accessory function in T cell activation. A diminished number of circulating T cells indicates the potential for significant immune compromise and is associated with doubling of the fatality rate.

Herpesviruses — Herpesvirus infections can cause transient depression of cell-mediated immunity manifested by decreased in vitro proliferation with mitogens, and reduced interferon-gamma production in response to mitogens during the acute phase of the illness. These phenomena are most profound and long-lived with cytomegalovirus, but secondary superinfection is unusual. Herpesvirus persistence has also been implicated in the mechanisms of immunosenescence (see above).

Bacterial infections — Infection by bacteria is not generally associated with significant secondary immune suppression. One exception may be bacteria that produce "superantigen" toxins (eg, staphylococci, streptococci). Superantigens can bind simultaneously to MHC class II antigens and to the non-antigen-binding region of T cell receptor variable regions, thereby stimulating large numbers (up to 20 percent) of T cells. These T cells then produce large amounts of inflammatory cytokines, which lead to a syndrome resembling septic shock with multisystem organ failure (eg, staphylococcal toxic shock syndrome). Following interaction with superantigens, circulating T cells first increase, then decrease. Animal studies have shown that some T cells enter a state of anergy and cannot be further activated. These bacteria also produce superantigen like molecules with distinct biological activities, including interference with opsonophagocytosis and other neutrophil functions. Although these bacterial products are very important as virulence factors, their role in inducing any secondary immune suppression is unclear.

Mycobacterial infections — Mycobacteria establish chronic infections and replicate within phagocytic cells (monocytes and macrophages). Several secreted and surface mycobacterial products inhibit the ability of the infected cell to kill the invader and also prevent normal cooperation with other cells in immune responses. This may lead to some increase in the risk of secondary infection.

Parasite infestation — The immune suppression resulting from protozoan infestation tends to be more pronounced than that found with other classes of microbes, with the exception of HIV. As an

example, cell-mediated immunity is generally suppressed in malaria. This leads to susceptibility to infections by other microbes, delayed graft rejection, and to a higher rate of various malignancies.

Some of the possible mechanisms underlying the immune suppression occurring during parasitic infection include:

- Alteration in macrophage function
- The induction of suppressor T cells
- Production of immunosuppressive factors by the parasites themselves, which may promote the first two mechanisms or may affect other aspects of immune function

A decreased capacity for antigen presentation and microbicidal activity has been demonstrated in macrophages in malaria, trypanosomiasis, and leishmaniasis. Leishmaniasis is also associated with diminished macrophage expression of MHC class II and interleukin-1 production, while the function of normal T cells may be suppressed when cultured together in malaria and trypanosomiasis.

Suppressor T cells have been implicated in the immune dysfunction in many parasitic diseases, but a detailed description of their phenotype and function is lacking. Similarly, many studies have demonstrated the presence of factors in parasite culture fluids that may nonspecifically suppress lymphocyte proliferation or may activate B cells polyclonally, leading to autoantibody production. The chemical characteristics and function of any of these factors has not yet been determined.

Malaria — Malaria infection is one aspect of the marked association of Epstein-Barr virus (EBV) infection with Burkitt lymphoma that is observed in Africa, but not in Europe or America. Although the seroprevalence of EBV in western countries is significant, malaria is uncommon. Plasmodia inhibit the ability of cytotoxic T cells to maintain EBV transformed B cells under control, leading to lymphomas.

Other parasites — Delayed graft rejection and impaired humoral immunity have also been found in infestations with helminths, such as Trichinella and schistosomes. Infection with Trypanosoma brucei is associated with diminished antibody responses, cutaneous anergy, and diminished in vitro T cell mitogen responses.

THEME №7. PRINCIPLES OF TRANSPLANTATION IMMUNITY. IMMUNOLOGY OF REPRODUCTION. IMMUNODEPENDENT FORMS OF INFERTILITY

TRANSPLANTATION IMMUNOBIOLOGY

Clinical transplantation encompasses the transplantation of organs and islets of Langerhans, in which it is necessary to overcome the host-versus-graft (HVG) immune response to avoid rejection, as well as hematopoietic cell transplantation, in which not only the HVG but also the graft-versus-host (GVH) immune response must be contended with. Because preparations of marrow or mobilized peripheral blood stem cells contain mature T cells, their administration to conditioned, and consequently immunoincompetent, recipients are associated with the risk of GVH disease. Organs transplanted include the cornea, kidney, liver, heart, lung, small intestine, and pancreas, and even the hand and face. The list of transplanted allogeneic cells is likely to expand in the future to include other cell types such as hépatocytes, myoblasts, and stem cell-derived replacement cells. Transplants originating from a member of the same species are referred to as allotransplants. However, many believe that transplants from other species, termed xenografts, represent a promising solution to the severely inadequate supply of allogeneic organs and tissues, and such grafts may be used in the future.

Transplantation may be:

- ***Autologous*** – the individual's own tissue, usually bone marrow or haemopoietic stem cells are returned to the body.
- ***Syngeneic*** – tissue is transplanted from a genetically identical individual (identical twin).

- ***Allogeneic*** – tissue is transplanted from a non-identical individual, from the same species.
- ***Xenogeneic*** – tissue is transplanted from one species to another.

Rejection is not a risk in autologous and syngeneic transplantation but is a major problem in allogeneic transplantation. Xenotransplantation carries a high risk of rejection, which together with concerns about infection have prevented clinical trials.

Clinical transplantation may be divided into haemopoietic stem-cell (HSC) transplantation, solid organ transplantation and tissue transplantation.

HSCs are progenitor cells that develop into the cellular elements of blood and the immune system. Until recently, bone marrow was the only viable source of HSCs, however, these cells are now harvested from peripheral blood after mobilisation by growth factors, and from umbilical cord blood.

Solid organ transplantation is used to treat irreversible failure of kidneys, liver, heart, lung and pancreas. Small bowel transplantation remains experimental.

Tissue transplantation refers to transplantation of non-vascularised tissues. The lack of blood-supply protects from rejection. However, when tissues expressing HLA-antigens are

transplanted, sensitisation may result. Tissues routinely transplanted include cornea, bone and human and porcine heart valves. Islet cell transplantation has been successfully performed, and techniques are being refined.

IMMUNOLOGICAL ASPECTS HSC transplantation replaces the bone marrow and immune system of the recipient. The recipient's body is foreign to the new donor immune system, which may mount an attack on this 'foreign' tissue. This is termed graft-versus-host disease (GvHD).

In solid organ transplantation, the donor organ is foreign to the recipient's immune system. Rejection occurs when the recipient's immune system attacks the transplanted organ.

Non-vascularised tissue is generally not rejected, and systemic immunosuppression is not required. However, a corneal graft that becomes vascularised may be rejected.

HAEMOPOIETIC STEM CELL TRANSPLANTATION

INDICATIONS Pluripotential haemopoietic stem cell (HSC) transplantation refers to a range of procedures that restore haemopoietic function. Bone marrow, peripheral blood and umbilical cord blood are sources of HSCs. Indications for HSC grafting are many and include:

♦***Haematological malignancies***

— lymphoid - acute lymphoblastic leukaemia (ALL), if it falls within a poor prognostic category or for relapsing disease

— Hodgkin's and non-Hodgkin's lymphomas (relapsed; high grade) multiple myeloma (young, poor prognosis)

— myeloid - acute myeloid leukaemia (AML) - (1st/2nd remission); chronic myeloid leukaemia.

♦***Primary haematological diseases***

— aplastic anaemia
— thalasaemias
— sickle-cell disease
— myelodysplasia.

♦***Primary Immunodeficiencies***

— SCIDs
— progressive combined immunodeficiencies
— severe neutrophil-related immunodeficiencies (CGD and LAD).

♦***Inborn errors of metabolism***

— Gaucher's disease

— mucopolysaccharidoses (Hurler's)
— osteopetrosis
— leucodystrophies.

♦***Solid organ malignancies*** - rescue from marrow-ablating chemotherapy (breast; lung; testis; ovary; Ewing's sarcoma; neuroblastoma)

♦***Autoimmune disorders*** - experimental in severe, therapy-resistant conditions (scleroderma etc.).

TYPES OF TRANSPLANT Haemopoietic transplants are either autologous or allogeneic. Autologous HSC transplantation is relatively straightforward, however, allogeneic HSC transplantation is more challenging, and may be complicated by:

♦Non-engraftment (recipient rejects donors cells, which never 'take')

♦ Graft -versus-Host Disease.

Autologous transplantation

The use of auto-grafts is increasing because of the lack of rejection and GvHD complications as well as quicker marrow recovery. This type of transplant is suitable in:

♦Haematological malignancies - stem cells obtained during remission are used to restore marrow function following intensive, marrow-ablating chemotherapy.

♦Non-haematological malignancy - restoration of marrow function following ablative therapy.

♦Autoimmune disease - currently limited to clinical trials.

Transplantation of residual tumour cells is a risk. Increasingly sensitive detection and eradication of minimal residual disease from stem cell preparations has increased success rates.

Allogeneic transplantation

Allografts are used when there is an intrinsic fault in HSCs.

♦Congenital abnormalities - immunodeficiency, haemoglobinopathies and metabolic disorders (e.g. mucopolysaccharidoses). HSC transplantation is not used if the risks of the transplant procedure outweigh the benefit of correcting the underlying disease (e.g. X-linked agammaglobulinaemia).

♦Some haematological malignancies - for example, CML, myelodysplasia, aplastic anaemia. Immune non-identity may have some positive effects. Graft recognition and elimination of leukaemic cells (graft versus leukaemia effect), reduces relapse rate.

While allogeneic transplants may offer cure (SCID) or improved outlook in very serious conditions (CML, aplastic anaemia), there are significant risks as outlined:

♦Rejection - failure of engraftment is more common than in autologous transplantation.

♦GvHD may be acute, occurring immediately post-transplant phase or chronic. Preventive measures include limitation of marrow mismatch, pre-treatment of stem cell transplant (T cell depletion) and immunosuppression of the host (Figure 7.1).

The following complications occur after both autologous and allogeneic HSC transplantation, but are more common and severe with allografts:

♦Severe marrow dysfunction, is more prolonged in allogeneic transplants, requiring intensive transfusion support and infection prevention measures (isolation, reverse- barrier nursing, antibiotic prophylaxis). Severe mucositis and alopoecia are almost inevitable. Bacterial, fungal, and certain viral infections occur frequently. CMV-negative donors are preferable and anti-viral prophylaxis is required if the donor was CMV- positive.

♦Immunodeficiency is profound immediately post-transplant and some compromise is common for up to two years. In SCID, T cell restoration usually occurs, however, if B cells do not reconstitute long-term antibody support is required. Immune reconstitution is delayed and deficient in patients with GvHD.

♦Veno-occlusive disease - occurs with pre-existing liver disease, intensive conditioning, or if undergoing second transplant.

♦Secondary malignancies - skin; EBV-related lymphomas.

♦Endocrine - infertility.

♦Psychological - symptoms are common.

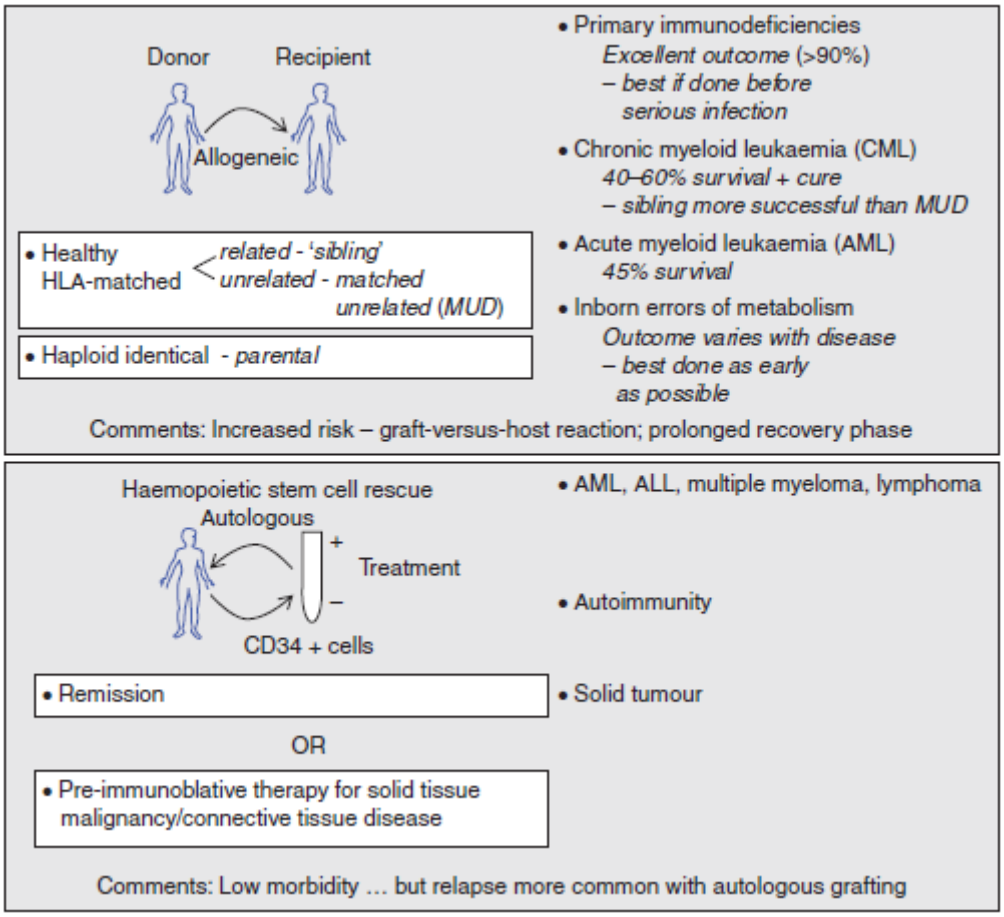

Figure 7.1 Bone marrow transplantation.

Sources of HSCs HSCs are obtained from:

♦ Bone marrow - aspirated under general anaesthetic, from puncture of the iliac crests.

♦ Peripheral blood - a viable and convenient source of HSC, particularly with improved cell harvesting and separation techniques. Treating the donor with haemopoietic growth factors increases numbers of HSCs. The outcome post-grafting with PBSCs is often better than with bone marrow transplantation.

♦Cord blood - is a rich source of immature stem cells of limited immunogenicity. The number of cells that can be retrieved limits this application.

Donors

All potential allogeneic donors must:

♦Give informed consent.

♦Undergo screening for blood-borne infections.

♦Undergo HLA typing - The ideal but rarely available donor is an HLA-matched sibling. HLA-matched unrelated donors (MUD transplants), usually identified from bone-marrow registries, are a less-favoured option. Haploid-identical matching (parental donors will share one HLA haplotype) has been successful in SCID, as rejection is not a problem.

Incompletely matched grafts can be assessed prior to transplantation for the likelihood of problems using a number of specialised immunological tests (mixed lymphocyte reaction, and precursor lymphocyte frequency measurement).

In autologous transplantation, HSC must be harvested during clinical remission. Outlook for graft recipients is optimised by:

♦Appropriate pre-transplant conditioning (enough to eradicate diseased cells and to make enough physical space for the new bone marrow to develop)

♦Aggressive infection prophylaxis and treatment

♦Good supportive care.

GvHD prophylaxis is given if there is a significant risk. The underlying condition also influences outcome. SCID patients generally do very well even with haploidentical donors. SCID patients are usually managed at specialist immunodeficiency transplant centres.

SOLID ORGAN TRANSPLANTATION

The most commonly transplanted solid organ is the kidney, however liver, heart, lung and pancreas transplantation are routine treatments for irreversible failure of these organs. Islet cell transplantation techniques are being refined, and small bowel transplantation remains experimental treatment.

INDICATIONS General indications for solid organ transplantation are:

♦Irreversible organ failure

♦Underlying condition with acceptable risk of recurrence

♦Recipient free from infection and malignancy

♦Recipient fit for major surgery.

TRANSPLANT ASSESSMENT Patients are thoroughly assessed before going on the waiting list to ensure:

♦Medical and surgical fitness for transplantation

♦Psychologically suitable for surgery and post-transplantation therapy

♦Understanding of the process and willingness to give informed consent.

Laboratory assessment includes viral studies, blood grouping, HLA typing and identification of anti-HLA antibodies which could damage grafts. Anti-HLA antibodies develop when individuals are exposed to foreign HLA antigens. HLA sensitising events include pregnancy, blood transfusions and previous transplants.

PREPARATION FOR TRANSPLANTATION The waiting time depends on the number of patients awaiting transplantation, clinical priority for the patient and the donor rate in the organ sharing area. Living-related (or unrelated) kidney transplantation may be considered, and is associated with superior outcomes for the recipient. Organ allocation policies vary between organs - size is a major determinant in thoracic transplantation, while HLA matching receives more attention in renal transplantation. A graft bearing HLA-antigens to which a patient has antibodies is not acceptable. Such anti-HLA antibodies will be identified at initial or ongoing assessments, well in advance of a donor becoming available.

When a patient is considered for an organ, cross-matching is performed as a final check that the patient does not have anti-donor-HLA antibodies, which would cause hyperacute rejection. If cross-match results are acceptable, surgery may proceed. Every effort is made to minimise the ischaemic time of the organ. In the case of heart transplants acceptable ischaemia is limited to 4 hours, whereas kidneys can withstand over 24 hours in appropriate conditions. Longer ischaemia is associated with inferior outcomes.

COMPLICATIONS Patients experience different complications at different times post transplantation. The prevalence of each complication depends on the organ transplanted and in some cases, the patient's primary disease. Problems include:

♦Surgical - bleeding, anastomotic leaks, wound infection

♦Thrombosis of arteries and veins

♦Rejection

♦Infection

♦Malignancy - post-transplant lymphoproliferative disorder (PTLD); skin cancers; other tumours

♦Drug toxicity - steroid-induced osteoporosis; hirsutism and gingival hypertropy with cyclosporin

♦Recurrence of primary disease in transplanted organ

♦Rejection is a common occurrence following transplantation as the immune system attacks the graft (non-self).

REJECTION: WHAT MOLECULES DOES THE IMMUNE SYSTEM SEE?

The immune system responds to molecules that differ between the donor and recipient.

These may be divided into:

♦Major antigens - HLA (MHC) antigens, which are recognised by the immune system without processing, and elicit strong cellular and antibody responses. Routine typing and cross-matching usually identifies these antigens.

♦Minor antigens - numerous molecules, not identified by tissue typing techniques. Minor antigens require processing, and are presented to the recipient's immune system complexed with recipient HLA molecules (indirect presentation). They elicit T cell responses, weaker than those elicited by HLA molecules.

Classification of rejection Classification of rejection is based on the mechanism of tissue damage - different types being common at different times post transplantation (Table 7.1).

Table 7.1 Types of rejection

TYPE OF REJECTION	TIME POST Tx	MECHANISM OF TISSUE DAMAGE
Hyperacute	<24 hours	Preformed antibody, complement, coagulation
Acute cellular	Common < 6 months	Cytotoxic T cells
Acute humoral	Common < 6 weeks	Antibody produced post-transplant
Chronic	Months-years	Uncertain. Scarring in response to injury

MECHANISMS OF GRAFT DAMAGE

ANTIBODY-MEDIATED REJECTION Binding of antibody results in complement activation and deposition of the membrane attack complex (MAC). Graft endothelium becomes damaged, and the coagulation cascade is activated. Biopsy shows thrombi, neutrophil infiltration and severe tissue injury. DIF demonstrates deposition of antibody and complement. Anti-donor antibody is frequently detectable in the serum. Antibody mediated rejection occurs in about 1% of renal transplant recipients.

T CELL-MEDIATED REJECTION Acute cellular rejection T cells infiltrate the graft interstitium and renal tubules. Cytotoxic T cells directly injure the allograft, and cytokines produced by helper and cytotoxic T cells attract more inflammatory cells and up-regulate HLA antigen expression thus amplifying the process (Figure 7.2). Cellular rejection is seen in about 20% of renal allograft recipients.

Chronic rejection The mechanisms of chronic rejection are poorly understood. It appears that early damage to the graft (due to rejection, reperfusion injury, cytomegalovirus) initiates an inflammatory process leading to scarring and tissue remodelling. Virtually all long-surviving solid organ grafts show evidence of chronic rejection.

Episodes of rejection are a common complication, even with modern immunosuppression, in recipients of all solid organs.

The risk can be reduced by:

♦Optimal HLA matching (however, not possible for patients with rare HLA types)

♦Improved cross matching (reducing antibody-mediated rejection)

♦More potent immunosuppression (but also increases the risk of infection and malignancy).

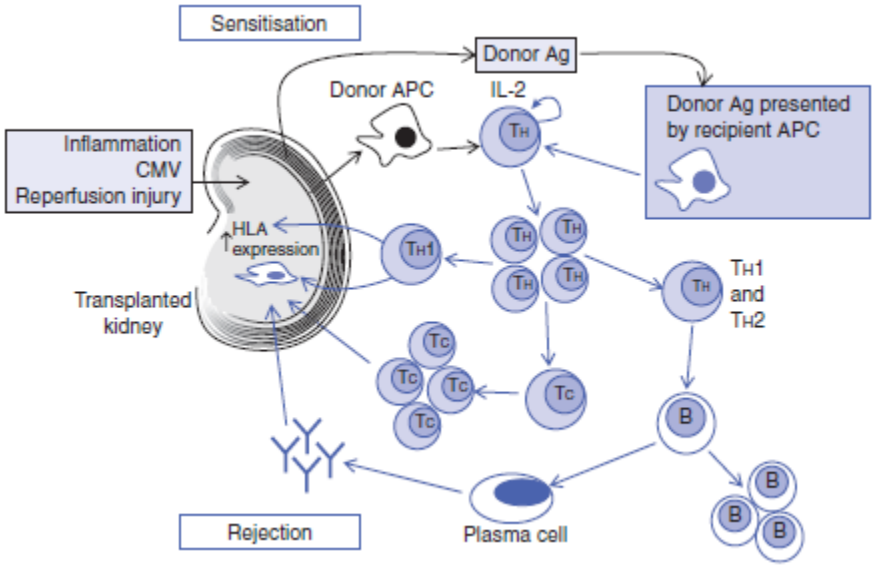

Figure 7.2 Mechanisms of graft rejection

SURVIVAL Graft survival is longest for kidneys, followed by heart, liver and lung (see Table 7. 2). Survival is affected by:

♦Donor factors (age, sex, cause of death)

♦Perioperative events (cold ischaemia time, surgical aspects)

♦Recipient factors (age, sex, comorbidity, sensitisation, HLA match, adherence to therapy).

Table 7. 2 Graft survival figures

ORGAN	KIDNEY	HEART	LUNG	LIVER
1 year graft survival	>87%	83%	70%	77%
Median survival	11 years	9 years	5 years	8 years

THERAPY Immunosuppression includes 3-4 agents, typically steroids, cyclosporin/tacrolimus and azathioprine/mycophenylate with additional antibody therapy added in high-risk patients. Acute cellular rejection is treated with high dose steroids, and occasionally T cell-depleting antibodies. Antibody-mediated rejection is treated with plasmapheresis to rapidly remove antibodies, and augmented immunosuppression. Currently there are no treatments for chronic rejection.

Theme №8. TUMOR IMMUNOLOGY. IMMUNE ASPECTS OF AUTOIMMUNE DISEASE

MALIGNANT TRANSFORMATION

The proliferation of normal cells is carefully regulated. However, such cells when exposed to chemical careinogens, irradiation and certain viruses may undergo mutations leading to their transformation into cells that are capable of uncontrolled growth, producing a tumor or neoplasm.

A tumor may be:

- Benign, if it is not capable of indefinite growth and the host survives.
- Malignant, if the tumor continues to grow indefinitely and spreads (metastasizes), eventually killing the host. This uncontrolled growth may be due to up regulation of oncogenes (cancer-inducing genes) and/or down regulation of tumor suppressor genes (that normally inhibit tumor growth often by inducing cell death).

EVIDENCE FOR IMMUNE REACTIVITY TO TUMORS

There is a lot of evidence that tumors can elicit an immune response. Such evidence includes:

- Tumors that have severe mononucleareell infiltration have a better prognosis than those that lack it.
- Certain tumors regress spontaneously (e.g., melanomas, neuroblastomas), suggesting an immunological response.
- Some tumor metastases regress after removal of primary tumor which reduces the tumor load, thereby inducing the immune system to kill the residual tumor.
- Although chemotherapy leads to rejection of a large number of tumor cells, the few tumor cells that evade the action of the drugs can outgrow and kill the host. However, the immune system may be able to mount an attack against the few tumor cells that are spared by the chemotherapeutic agent.
- There is an increased incidence of malignancies in immuno-deficient patients such as AIDS patients who are susceptible to Kaposi sareoma and transplant patients who are susceptible to Epstein Barr virus (EBV)-induced lymphoma.
- Tumor-specific antibodies and T lymphocytes (detected in cytotoxicity and proliferative response assays) have been observed in patients with tumors.
- The young and the old population have an increased incidence of tumors. These members of the population often have an immune system that is compromised.
- Hosts can be specifically immunized against various types of tumors demonstrating tumor antogens can elicit an immune response.

TUMOR ASSOCIATED ANTIGENS. In order for the immune system to react against a tumor, the latter must have antigens that are recognized as foreign. A number of alterations in gene expression occur in cells during tumorigenesis. Tumorigenesis may lead to expression of new antigens (neoantigens) or alteration in existing antigens that are found on normal cells. These antigens may include membrane receptors, regulators of cell cycle and apoptosis, or molecules involved in signal transduction pathways.

There are 2 main types of tumor antigens:

- Tumor-specific transplantation antigens (TSTA) which are unique to tumor cells and not expressed on normal cells. They are responsible for rejection of the tumor.
- Tumor associated transplantation antigens (TATA) that are expressed by tumor cells and normal cells.

Although chemical- ,UV- or virus-induced tumors express neo-antigens, the majority of these tumors are often weakly immunogenic or non-immunogenic. In most cases, TSTAs cannot be identified easily. Some of these antigens may be secreted while others may be membrane-associated molecules.

Tumor associated transplantation antigens (TATA)

The majority of tumor antigens are also present on normal cells and are referred to as tumor associated transplantation antigens. They may be expressed at higher levels on tumor cells when compared to normal cells. Alternatively, they may be expressed only during development of cells and lost during adult life but re-expressed in tumors.

Tumor-associated developmental antigens or onco-fetal antigens

These include alpha-fetoprotein (AFP) and careino-embryonic antigen (CEA) found secreted in the serum. AFP is found in patients with hepatocellulareareinoma whereas CEA is found in colon cancer. These are important in diagnosis.AFP is produced only as a secreted protein whereas CEA is found both on cell membranes and in secreted fluids. Since secreted antigens contribute little toward immunity against tumors, the role of these neo-antigens in immuno-surveillance is questionable.

The normal range of AFP concentrations in humans is 0-20 ng/ml. This level rises considerably in patients with hepatomas and non-seminal testicularareareinoma. A 5-fold or higher rise in this protein is used for monitoring hepatomas and testicularareancers. AFP level may also be raised in some non-malignant conditions, such as cirrhosis, in hepatitis and other forms of liver damage.

CEA levels in normal people range up to 2.5 ng/ml, but they increase significantly in certain malignancies, particularly colo-rectal cancers. They may also rise in some non-malignant conditions (such as chronic cirrhosis, pulmonary emphysema and heavy smoking). Levels that are 4 to 5 times normal have been used to predict recurrence of colo-rectal tumors.

TUMOR ASSOCIATED TRANSPLANTATION ANTIGENS ON VIRAL TUMORS

Viruses that cause human tumors include:

DNA viruses

- Papova (papilloma, polyoma) viruses: Papilloma virus causes cervical cancer.
- Hepatitis virus: Hepatitis B virus causes hepatocellulareancer.
- Adenoviruses may also be tumorigenic
- RNA viruses
- Retroviruses: Human T-lymphotropic viruses (HTLV-I and HTLV-II) causes T cell leukemias.

A number of viruses cause different types of tumors in animals (for example, SV-40 virus, adenovirus, Rous sareoma virus, Friend erythroleukemic virus, Moloney Rauscher and Gross viruses). Viruses are involved or suspected to be involved in some human malignancies (HTLV-1 in leukemia, hepatitis-B virus in hepatic careinoma, papilloma virus in cervical cancer). Virus-induced tumors express cell surface antigens (distinct from antigens of the virion itself) which are shared by all tumors induced by the same virus. These antigens are characteristic of the tumor-inducing virus, regardless of tissue origin of the tumor or animal species in which the tumor exists.

TUMOR ASSOCIATED TRANSPLANTATION ANTIGENS ON CHEMICALLY-INDUCED TUMORS. Chemically-induced tumors are different from virally-induced tumors in that they are extremely heterogeneous in their antigenic characteristics. Thus, any two tumors induced by the same chemical, even in the same animal, rarely share common tumor specific antigens. These unique antigens on chemically-induced tumors are referred to as tumor specific transplantation antigens (TSTA).

SYNGENEIC, ALLOGENEIC AND XENOGENEIC TUMORS. A tumor that grows in an animal strain will also grow in another animal belonging to the same inbred strain obtained by repeated brother-sister matings. These animals express the same MHC molecules and are referred to as syngeneic. However, most normal animal populations are allogeneic and have various MHC haplotypes. Thus, a tumor transferred from one animal to another animal belonging to an outbred strain is rejected because of the allo-MHC rather than the TSTA. A tumor transferred from an animal belonging to one species to another animal belonging to a different species is rapidly rejected because the animals are xenogeneic.

IMMUNITY AGAINST TUMORS. Although there is ample evidence for anti-tumor immune reactivity in humans, evidence for immunity against malignancy comes mostly from experimental studies with animals. In these, mice were immunized by administering irradiated tumor cells or following removal of a primary tumor challenged with the same live tumor. These animals were found to be resistant to rechallenge with the same live tumor. While antibodies may develop against few cancers, cell-mediated immunity plays a critical role in tumor rejection. Thus, immunity can be transferred, in most cases, from an animal, in which a tumor has regressed, to a naive syngeneic recipient by administration of T lymphocytes. The T helper (Th) cells recognize the tumor antigens that may be shed from tumors and internalized, processed and presented in association with class II MHC on antigen presenting cells. These

Th cells, when activated, will produce cytokines. Thus, the Th cells provide help to B cells in antibody production. Cytokines such as IFN-gamma may also activate macrophages to be tumoricidal. Furthermore, the Th cells also provide help to tumor-specific cytotoxic T cells (CTLs) by inducing their proliferation and differentiation. The CTLs recognize tumor antigens in the context of class I MHC and mediate tumor cell lysis. In tumors that exhibit decreased MHC antigens, natural killer (NK) cells are important in mediating tumor rejection.

ESCAPE FROM IMMUNO-SURVEILLANCE. According to the Immune Surveillance Theory, cancer cells that arise in the body are eliminated by the immune system. However, due to impaired immune reactivity, cancer cells may escape destruction. Tumors evade immune recognition by several mechanisms. Tumors may not express neo-antigens that are immunogenic or they may fail to express co-stimulatory molecules required for the activation of T cells. In addition, certain tumors are known to lack or be poor expressers of MHC antigen. Another reason for failure of immune surveillance may be the fact that in the early development of a tumor, the amount of antigen may be too small to stimulate the immune system (low dose tolerance) or, due to the rapid proliferation of malignant cells (high dose tolerance), the immune system is quickly overwhelmed. In addition, some tumors may evade the immune system by secreting immunosuppressive molecules and others may induce regulatory cells particularly the CD4+CD25+ FoxP3+ T regulatory cells. Also, some tumors may shed their antigens which in turn may interact and block antibodies and T cells from reacting with the tumor cells.

USE OF TUMOR NEO-ANTIGENS IN PATIENT MANAGEMENT. The presence of neo-antigens on tumor cells has been exploited for both diagnostic and therapeutic purposes.

Immuno-diagnosis. Monoclonal antibodies labeled with radioisotope have been used for in vivo detection of relatively small tumor foci. Antibodies have also been used in vitro to identify the cell origin of undifferentiated tumors, particularly of lymphocytic origin. Also, immuno-histological staining is used to confirm suspected metastatic foci, especially in bone marrow.

Immunotherapy. Immunotherapy has been used as a novel means of treating cancer. Both active and passive means of stimulating the non-specific and specific immune systems have been employed, in some cases with significant success (Table 8. 1).

Table 8. 1 Immunotherapy of tumors

active	**non-specific**	BCG, *Propionibacterium acnes*, levamisole, cytokine genes, *etc.*
	specific	Killed tumor cells or their extract, recombinant antigens, idiotype, co-stimulatory molecule genes, *etc*.
passive	**nonspecific**	LAK cells, cytokines
	specific	Antibodies alone or coupled to drugs, pro-drug toxins or radioisotope; bispecific antibodies; T-cells
	combined	LAK cells and bispecific antibody

* BCG: Bacillus Calmette Geurin is a bovine strain of Mycobacterium tuberculosis

Active Immunotherapy

In this, the host actively participates in mounting an immune response

- Specific activation is achieved by using vaccines: e.g. Hepatitis B vaccine and Human Papilloma virus (HPV) vaccine
- Non-specific activation is achieved by immunization with, for example, Bacillus Calmette-Guerin (BCG) and Corynebacterium parvum

These activate macrophages to be tumoricidal.

Passive Immunotherapy

This involves transfer of preformed antibodies, immune cells and other factors into the hosts.

Specific:

1) Antibodies against tumor antigens (e.g. Her2/Neu for treatment of breast cancer)
2) Antibodies against IL-2R for Human T lymphotropic virus (HTLV-1)-induced adult T cell leukemia
3) Antibodies against CD20 expressed on non-Hodgkin's B cell lymphoma. These antibodies bind to tumor antigens on the cell surface and activate complement (C') to mediate tumor cell lysis. In addition, Fc receptor bearing cells such as NK cells, macrophages and granulocytes may bind to the antigen-antibody complexes on the tumor cell surface and mediate tumor cell killing through antibody-dependent cell-mediated cytotoxicity.
4) Antibodies conjugated to toxins, radioisotopes and anti-cancer drugs have also been used. These enter the cells and inhibit protein synthesis. e.g. anti-CD20 conjugated to Pseudomonas toxin or ricin toxin.

There are several problems with the use of antibodies

- Antibodies are not efficient because the tumor antigens are associated with class I MHC antigens.
- Tumors may shed antigen or antigen-antibody complexes. Thus, immune cells cannot mediate tumor destruction.
- Some antibodies may not be cytotoxic.
- Antibodies may bind non-specifically to immune cells expressing the Fc receptors which include NK cells, B cells, macrophages and granulocytes without binding to tumor cells.

Nonspecific:

1) Adoptive Transfer of lymphocytes:
   - Lymphokine-activated killer (LAK) cells which are IL-2 activated T and NK cells.
   - Tumor-infiltrating lymphocytes (TIL)
2) Dendritic cells pulsed with tumor antigens may induce tumor-specific T cell responses. As tumor Ags are usually not known, tumor lysates are used.
3) Cytokines
   - IL-2: Activates T cells/NK cells expressing IL-2 receptors. This is used in the treatment of renal cell careinoma and melanoma
   - IFN-alpha: This induces MHC expression on tumors and used in the treatment of hairy B cell leukemias
   - IFN-gamma: This increases class II MHC expression; used in the treatment of ovarian cancers.
   - TNF-alpha: This kills tumor cells.
4) Cytokine gene transfected tumor cells may also be used which can activate T or LAK cell-mediated anti-tumor immunity.

A variety of immunopotentiating agents (biological response modifiers) are used to enhance anti-tumor immunity. They include bacterial products, synthetic chemicals and cytokines (Table 8. 2). Most of these agents exert their effects by activating macrophages and natural killer (NK) cells, eliciting cytokines or enhancing T-cell functions.

A number of cytokines have been used to potentiate the immune function of the host since the discovery that these cytokines have potent and selective effects on certain components of the immune system (Table 8. 3).

Table 8. 2 Non-specific active immunotherapy: biological response modifiers (BRMs)

Type of BRM	Examples	Major effect
Bacterial product	BCG, *P. acnes*, muramyl di-peptide, trehalose dimycolate	Activate macrophages and NK cells (via cytokines)
Synthetic molecules	pyran, poly I:C, pyrimidines	Induce interferon production
Cytokines	interferon-alpha, -beta, -gamma, IL-2, TNF	Activate macrophages and NK cells

Table 8. 3 Cytokine therapy of tumors

Cytokine	Tumor type and result	Anti-tumor mechanism(s)
IFN-alpha, beta	Remission of hairy cell leukemia, weak effect on some careinomas	Increased expression of class I MHC, possible cytostatic anti-tumor effect,
IFN-gamma	Remission of peritoneal careinoma of ovary: ineffective systemically	Increased MHC antigens; macrophage, Tc and NK cell activation
IL-2	Remission in renal careinoma and melanoma	T-cell proliferation and activation, NK cells activation
TNF-alpha	Can reduce malignant ascites	Macrophage and lymphocyte activation

TOLERANCE AND AUTOIMMUNITY

Tolerance refers to the specific immunological non-reactivity to an antigen resulting from a previous exposure to the same antigen. While the most important form of tolerance is non-reactivity to self antigens, it is possible to induce tolerance to non-self antigens. When an antigen induces tolerance, it is termed tolerogen.

TOLERANCE TO SELF ANTIGENS

We normally do not mount a strong immune response against our own (self) antigens, a phenomenon called self-tolerance. When the immune system recognizes a self antigen and mounts a strong response against it, autoimmune disease develops. Nonetheless, the immune system has to recognize self-MHC to mount a response against a foreign antigen. Thus, the immune system is constantly challenged to discriminate self vs non-self and mediate the right response.

INDUCTION OF TOLERANCE TO NON-SELF. Tolerance can also be induced to non-self (foreign) antigens by modifying the antigen, by injecting the antigen through specific routes such as oral, administering the antigen when the immune system is developing, etc. Certain bacteria and viruses have devised clever ways to induce tolerance so that the host does not kill these microbes. Ex: Patients with lepromatous type of leprosy do not mount an immune response against Mycobacterium leprae.

TOLERANCE TO TISSUES AND CELLS. Tolerance to tissue and cell antigens can be induced by injection of hemopoietic (stem) cells in neonatal or severely immunocompromised (by lethal irradiation or drug treatment) animals. Also, grafting of allogeneic bone marrow or thymus in early life results in tolerance to the donor type cells and tissues. Such animals are known as chimeras. These findings are of significant practical application in bone marrow grafting.

TOLERANCE TO SOLUBLE ANTIGENS. A state of tolerance to a variety of T-dependent and T-independent antigens has been achieved in various experimental models. Based on these observations it is clear that a number of factors determine whether an antigen will stimulate an immune response or tolerance (Table 8. 4).

IMMUNOLOGIC FEATURES OF TOLERANCE Tolerance is different from non-specific immunosuppression and immunodeficiency. It is an active antigen-dependent process in response to the antigen. Like immune response, tolerance is specific and like immunological memory, it can exist in T-

cells, B cells or both and like immunological memory, tolerance at the T cell level is longer lasting than tolerance at the B cell level.

Table 8. 4 Factors that determine induction of immune response or tolerance following challenge with antigen

Factors that affect response to Ag	Favor immune response	Favor tolerance
Physical form of antigen	Large, aggregated, complex molecules;	Soluble, aggregate-free, relatively smaller, less complex molecules, Ag not processed by APC or processed by cell without class II MHC
Route of Ag administration	Sub-cutaneous or intramuscular	Oral or sometimes intravenous
Dose of antigen	Optimal dose	Very large (or sometime very small) dose
Age of responding animal	Older and immunologically mature	Newborn (mice), immunologically immature
Differentiation state of cells	Fully differentiated cells; memory T and memory B cells	Relatively undifferentiated: B cells with only IgM (no IgD), T cells (*e.g.*cells in thymic cortex)

Induction of tolerance in T cells is easier and requires relatively smaller amounts of tolerogen than tolerance in B cells. Maintenance of immunological tolerance requires persistence of antigen. Tolerance can be broken naturally (as in autoimmune diseases) or artificially (as shown in experimental animals, by x-irradiation, certain drug treatments and by exposure to cross reactive antigens).

Tolerance may be induced to all epitopes or only some epitopes on an antigen and tolerance to a single antigen may exist at the B cell level or T cell level or at both levels.

MECHANISM OF TOLERANCE INDUCTION. The exact mechanism of induction and maintenance of tolerance is not fully understood. Experimental data, however, point to several possibilities.

Clonal deletion. T and B lymphocytes during development come across self antigens and such cells undergo clonal deletion through a process known as apoptosis or programmed cell death. For example, T cells that develop in the thymus first express neither CD4 nor CD8. Such cells next acquire both CD4 and CD8 called double-positive cells and express low levels of αβ TCR. Such cells undergo positive selection after interacting with class I or class II MHC molecules expressed on cortical epithelium. During this process, cells with low affinity for MHC are positively selected. Unselected cells die by apoptosis, a process called "death by neglect". Next, the cells loose either CD4 or CD8. Such T cells then encounter self-peptides presented by self MHC molecules expressed on dendritic cells. Those T cells with high affinity receptors for MHC + self-peptide undergo clonal deletion also called negative selection through induction of apoptosis. Any disturbance in this process can lead to escape of auto-reactive T-cells that can trigger autoimmune disease. Likewise, differentiating early B cells when they encounter self-antigen, cell associated or soluble, undergo deletion. Thus, clonal deletion plays a key role in ensuring tolerance to self antigen.

Peripheral tolerance. The clonal deletion is not a fool proof system and often T and B cells fail to undergo deletion and therefore such cells can potentially cause autoimmune disease once they reach the peripheral lymphoid organs. Thus, the immune system has devised several additional check points so that tolerance can be maintained.

Activation-induced cell death. T cells upon activation not only produce cytokines or carryout their effector functions but also die through programmed cell death or apoptosis. In this process, the death receptor (Fas) and its ligand (FasL) play a crucial role. Thus, normal T cells express Fas but not FasL. Upon activation, T cells express FasL which binds to Fas and triggers apoptosis by activation of caspase-

8. The importance of Fas and FasL is clearly demonstrated by the observation that mice with mutations in Fas (lpr mutation) or FasL (gld mutation) develop severe lymphoproliferative and autoimmune disease and die within 6 months while normal mice live up to 2 years. Similar mutations in these apoptotic genes in humans leads to a lymphoproliferative disease called autoimmune lymphoproliferative syndrome (ALPS).

Clonal anergy. Auto-reactive T cells when exposed to antigenic peptides on antigen presenting cells (APC) that do not possess the co-stimulatory molecules CD80 (B7-1) or CD86 (B7-2) become anergic (nonresponsive) to the antigen. Also, while activation of T cells through CD28 triggers IL-2 production, activation of CTLA4 leads to inhibition of IL-2 production and anergy. Also, B cells when exposed to large amounts of soluble antigen down-regulate their surface IgM and become anergic. These cells also up-regulate the Fas molecules on their surface. An interaction of these B cells with Fas-ligand bearing T cells results in their death via apoptosis.

Clonal ignorance. T cells reactive to self-antigen not represented in the thymus will mature and migrate to the periphery, but they may never encounter the appropriate antigen because it is sequestered in inaccessible tissues. Such cells may die out for lack of stimulus. Auto-reactive B cells, that escape deletion, may not find the antigen or the specific T-cell help and thus not be activated and die out.

Anti-idiotype antibody. These are antibodies that are produced against the specific idiotypes of other antibodies. Anti-idiotypic antibodies are produced during the process of tolerization and have been demonstrated in tolerant animals. These antibodies may prevent the B cell receptor from interacting with the antigen.

Regulatory T cells (Formerly called suppressor cells). Recently, a distinct population of T cells has been discovered called regulatory T cells. Regulatory T cells come in many flavors, but the most well characterized include those that express CD4+ and CD25+. Because activated normal CD4 T cells also express CD25, it was difficult to distinguish regulatory T cells and activated T cells. The latest researeh suggests that regulatory T cells are defined by expression of the forkhead family transcription factor Foxp3. Expression of Foxp3 is required for regulatory T cell development and function. The precise mechanism/s through which regulatory T cells suppress other T cell function is not clear. One of the mechanisms include the production of immunosuppressive cytokines such as TGF-β and IL-10. Genetic mutations in Foxp3 in humans leads to development of a severe and rapidly fatal autoimmune disorder known as Immune dysregulation, Polyendocrinopathy, Enteropathy, X-linked (IPEX) syndrome. This disease provides the most striking evidence that regulatory T cells play a critical role in preventing autoimmune disease.

Termination of tolerance. Experimentally induced tolerance can be terminated by prolonged absence of exposure to the tolerogen, by treatments which severely damage the immune system (x-irradiation) or by immunization with cross reactive antigens. These observations are of significance in the conceptualization of autoimmune **diseases.**

AUTOIMMUNITY

Autoimmunity can be defined as breakdown of mechanisms responsible for self tolerance and induction of an immune response against components of the self. Such an immune response may not always be harmful (e.g., anti-idiotype antibodies). However, in numerous autoimmune diseases it is well recognized that products of the immune system cause damage to the self.

EFFECTOR MECHANISMS IN AUTOIMMUNE DISEASES. Both antibodies and effector T cells can be involved in the damage in autoimmune diseases.

GENERAL CLASSIFICATION. Autoimmune diseases are generally classified on the basis of the organ or tissue involved. These diseases may fall in an organ-specific category in which the immune response is directed against antigen(s) associated with the target organ being damaged or a non-organ-

specific category in which the antibody is directed against an antigen not associated with the target organ (Table 8. 5).

Table 8. 5. Spectrum of autoimmune diseases, target organs and diagnostic tests

	Disease	Organ	Antibody to	Diagnostic Test
Organ-Specific	Hashimoto'sthyroiditis	Thyroid	Thyroglobulin, thyroid peroxidase (microsomal)	RIA, Passive, CF, hemagglutination
	Primary Myxedema	Thyroid	Cytoplasmic TSH receptor	Immunofluorescence (IF)
	Graves' disease	Thyroid		Bioassay, Competition for TSH receptor
	Pernicious anemia	Red cells	Intrinsic factor (IF), Gastric parietal cell	B-12 binding to IF immunofluorescence
	Addison's disease	Adrenal	Adrenal cells	Immunofluorescence
	Premature onset menopause	Ovary	Steroid producing cells	Immunofluorescence
	Male infertility	Sperm	Spermatozoa	Agglutination, Immunofluorescence
	Insulin dependent juvenile diabetes	Pancreas	Pancreatic islet beta cells	
	Insulin resistant diabetic	Systemic	Insulin receptor	Competition for receptor
	Atopic allergy	Systemic	beta-adrenergic receptor	Competition for receptor
	Myasthenia graves	Muscle	Muscle, acetyl choline receptor	Immunofluorescence, competition for receptor
	Goodpasture's syndrome	Kidney, lung	Renal and lung basement membrane	Immunofluorescence (linear staining)
	Pemphigus	Skin	Desmosomes	Immunofluorescence
	Pemphigoid	Skin	Skin basement membrane	Immunofluorescence
	Phacogenic uveitis	Lens	Lens protein	
	AI hemolytic anemia	Red cells Platelet	Red cells	Passive hemagglutination Direct Coomb's test
	Idiopathic thrombocytopenia		Platelet	Immunofluorescence
	Primary biliary cirrhosis	Liver	Mitochondria	Immunofluorescence
	Idiopathic neutropenia	Neutrophils	Neutrophils	Immunofluorescence
	Ulcerative colitis	Colon	Colon lipopolysaccharide	Immunofluorescence
	Sjogren's syndrome	Secretory glands	Duct mitochondria	Immunofluorescence
	Vitiligo	Skin Joints	Melanocytes (fig 6)	Immunofluorescence
	Rheumatoid arthritis	Skin, kidney, joints etc	IgG	IgG-latex agglutination
Non-organ Specific	Systemic lupuserythematosus	joints, etc.	DNA, RNA, nucleoproteins	RNA-, DNA-latex agglutination, IF (granular in kidney)

Diseases are listed from the most organ-specific (top) to the least specific (bottom)

GENETIC PREDISPOSITION FOR AUTOIMMUNITY

Studies in mice and observations in humans suggest a genetic predisposition for autoimmune diseases. Association between certain HLA types and autoimmune diseases has been noted (HLA: B8, B27, DR2, DR3, DR4, DR5 etc.).

ETIOLOGY OF AUTOIMMUNITY DISEASE. The exact etiology of autoimmune diseases is not known. However, various theories have been offered. These include sequestered antigen, escape of auto-reactive clones, loss of suppressor cells, cross reactive antigens including exogenous antigens (pathogens) and altered self antigens (chemical and viral infections).

Sequestered antigen. Lymphoid cells may not be exposed to some self antigens during their differentiation, because they may be late-developing antigens or may be confined to specialized organs (e.g., testes, brain, eye, etc.). A release of antigens from these organs resulting from accidental traumatic injury or surgery can result in the stimulation of an immune response and initiation of an autoimmune disease.

Escape of auto-reactive clones. The negative selection in the thymus may not be fully functional to eliminate self reactive cells. Not all self antigens may be represented in the thymus or certain antigens may not be properly processed and presented.

Lack of regulatory T cells. There are fewer regulatory T-cells in many autoimmune diseases.

Cross reactive antigens. Antigens on certain pathogens may have determinants which cross react with self antigens and an immune response against these determinants may lead to effector cell or antibodies against tissue antigens. Post streptococcal nephritis and carditis, anticardiolipin antibodies during syphilis and association between Klebsiella and ankylosing spondylitis are examples of such cross reactivity.

DIAGNOSIS. Diagnosis of autoimmune diseases is based on symptoms and detection of antibodies (and/or very early T cells) reactive against antigens of tissues and cells involved. Antibodies against cell/tissue associated antigens are detected by immunofluorescence. Antibodies against soluble antigens are normally detected ELISA or radioimmunoassay (see table above). In some cases, a biological /biochemical assay may be used (e.g., Graves diseases, pernicious anemia).

TREATMENT. The goals of treatment of autoimmune disorders are to reduce symptoms and control the autoimmune response while maintaining the body's ability to fight infections. Treatments vary widely and depend on the specific disease and symptoms: Anti-inflammatory (corticosteroid) and immunosuppressive drug therapy (such as cyclophosphamide, azathioprine, cyclosporine) is the present method of treating autoimmune diseases. Extensive researeh is being carried out to develop innovative treatments which include: anti-TNF alpha therapy against arthritis, feeding antigen orally to trigger tolerance, anti-idiotype antibodies, antigen peptides, anti-IL2 receptor antibodies, anti-CD4 antibodies, anti-TCR antibodies, etc.

MODELS OF AUTOIMMUNE DISEASES. There are a number of experimental and natural animal models for the study of autoimmune diseases. The experimental models include experimental auto-allergic encephalitis, experimental thyroiditis, adjuvant induced arthritis, etc.

THEME №9. ATOPIC DISEASES

Hypersensitivity reactions occur when the immune system mounts an excessive response to a stimulus. This includes an excessive response to an infectious agent or a response to selfantigen resulting

in autoimmune disease. More commonly, hypersensitivity refers to reactions against non-pathogenic environmental stimuli, such as house dust mite, pollens, foods and drugs.

Gell and Coombs originally classified hypersensitivity reactions in the 1960s, based on the immunological mechanisms involved. This classification remains a very useful framework for considering many aspects of immunopathology.

Many of the examples shown in Table 9.1 are predominantly due to a single type of hypersensitivity mechanism. In several diseases, more than one type of hypersensitivity mechanism may be involved, and different manifestations of the same disease may be due to different mechanisms. Joint destruction in rheumatoid disease is predominantly T cell mediated; however, rheumatoid vasculitis is immune complex mediated. Extrinsic allergic alveolitis (EAA) is a chronic lung disease caused by hypersensitivity to a variety of inhaled antigens. Common examples of EAA include farmers' lung (due to hypersensitivity to Micropolyspora faeni spores), and pigeon fanciers' lung (hypersensitivity to proteins in pigeon droppings). However, hypersensitivity to a long list of antigens have been implicated in this disorder. Most patients have precipitating antibodies to the offending antigen, which led to the theory that EAA was due to Type III hypersensitivity with immune complex formation in the lung when inhaled antigen complexed with precipitating antibody. However, histology of affected lungs showed granulomatous inflammation, indicating the involvement of T cells (Type IV hypersensitivity). Type IV hypersensitivity is now thought to be the major cause of lung damage in EAA.

Table 9.1 Classification of hypersensitivity reactions.

TYPE AND MECHANISM	ENVIRONMENTAL STIMULI	SELF ANTIGENS	INFECTIOUS TRIGGERS
Type I: Immediate hypersensitivity	House dust mite Cat dander Foods (peanut) Drugs	None known	Schistosomiasis
Type II: Antibody-mediated cytotoxicity	Drug-induced immune haemolytic anaemia	Goodpasture's syndrome, Myasthenia gravis, Graves' disease	Infection-induced haemolytic anaemia, Rheumatic fever
Type III: Immune complex deposition	Serum sickness	Systemic lupus erythematosus	Post-infectious glomerulonephritis, Hepatitis C
Type IV: Delayed hypersensitivity (cellular)	Contact hypersensitivity, for example, Nickel	Rheumatoid arthritis, Hashimoto's thyroiditis	Hepatitis B, Tuberculoid leprosy

Most types of immunopathology can be classified according to the above scheme, however in some disorders other mechanisms of tissue damage may occur. The mechanism of tissue damage in ANCA-associated vasculitis probably involves Type IV hypersensitivity, however additional novel mechanisms may be present. Similarly cytokine release syndromes induced by pathogens (toxic shock syndrome) or drugs (OKT3) represent additional mechanisms of tissue injury (Figure 9.1).

TYPE 1 HYPERSENSITIVITY Type I hypersensitivity results in rapid clinical manifestations, and underlies many disorders widely recognised as 'allergies' such as hay fever and asthma. In individuals predisposed to Type I hypersensitivity, antigen exposure leads to IgE production. IgE binds Fc receptors on mast cells, packed with granules containing histamine and other preformed mediators. IgE cross-linking by allergen causes degranulation of mast cells and rapid release of mediators.

TYPE II HYPERSENSITIVITY Type II hypersensitivity is caused by cytotoxic antibodies binding to components of cells or tissues or antigen/hapten which has become intimately associated with cells. Usually IgG is involved and complement activation follows. Anaphylatoxins are produced and attract inflammatory cells to the site of antibody binding. In Goodpasture's disease antibodies to

glomerular basement membrane (GBM) bind to basement membrane in kidney and lung. Bound antibody cannot be removed by phagocytosis and intense inflammation results.

Antibodies may bind and affect the function of key molecules on a cell as in myasthenia gravis where antibodies bind to acetylcholine receptors at the motor end plate, reducing the ability of nerve impulses to activate muscles. Antibodies may activate cells; in Graves' disease antibodies bind to the TSH receptor and induce thyroxine production leading to hyperthyroidism.

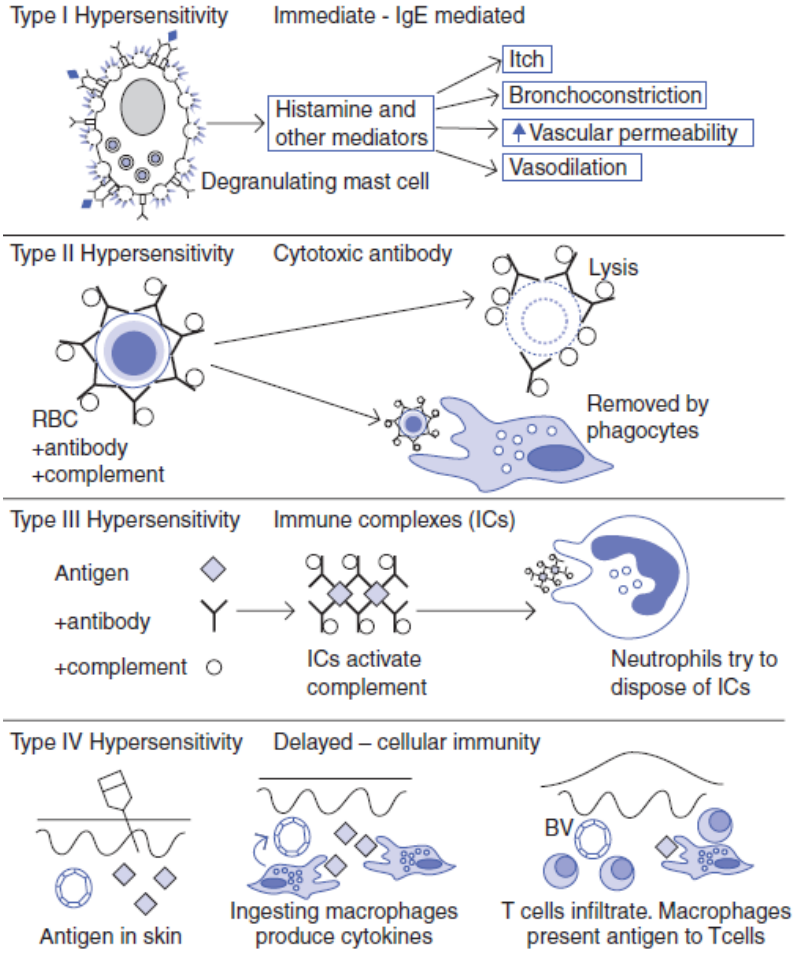

Figure 9.1 Mechanisms of hypersensitivity.

TYPE III HYPERSENSITIVITY Type III hypersensitivity results from formation or deposition of immune complexes (ICs) in tissues. ICs may be formed in the blood and trapped in tissues, or may be formed in situ if soluble antigen binds autoantibody. When antigen binds antibody, complement is activated and neutrophils and other inflammatory cells are attracted. Phagocytic cells ingest the ICs, however, when the capacity to clear complexes is exceeded, inflammation may occur.

Immune complex formation occurs in healthy individuals during infections and even after eating. However, in health immune complexes are cleared and do not cause inflammation. Antibody binding to antigen activates complement, and C3 cleavage products become incorporated into the immune complex lattice. C3 fragments bind to C3 receptors on red blood cells (RBCs), which then carry the immune complexes. RBCs move in the centre of blood vessels due to laminar flow of blood, and hence immune complexes are not normally in contact with blood vessel walls. In the spleen, phagocytic cells remove and ingest the immune complexes without damaging the RBCs. However, immune complexes may cause disease when this system is overwhelmed by excessive production, or when the capacity to dispose of immune complexes is reduced by complement deficiency or reduced numbers of complement receptors.

Additionally immune complexes formed in situ depend on the clearance capacity of local macrophages. If the capacity of resident macrophages is exceeded, inflammation results.

TYPE IV HYPERSENSITIVITY Type IV hypersensitivity is due to activation of cellular immunity and the onset of clinical manifestations is typically delayed by 48-72 hours. Antigen is taken up by antigen presenting cells (APCs), which then migrate to regional lymph nodes. Following antigen processing, APCs present antigen to responsive T cells, which proliferate and mature. Antigen specific TH1 cells then migrate to the periphery and when they encounter antigen are further stimulated. They secrete IFN-y which activates macrophages, and both T cells and macrophages contribute to the inflammatory process. When antigen is persistent, this process can result in granulomatous inflammation.

The Gell and Coombs classification of hypersensitivity is a useful framework to use when considering many aspects of immunopathology, however, more than one mechanism may be relevant to a single disease. Additionally other methods of tissue injury may occur.

ATOPY AND ALLERGIC INFLAMMATION

Atopy is a predisposition to generate IgE-mediated responses to environmental allergens. Atopy has a significant genetic component, inheritance being polygenic. When both parents are atopic over 50% of their children will also be atopic, however, 10% of children of nonatopic parents are atopic. The prevalance of atopy has increased rapidly, even within a single generation. This implies significant environmental influences.

Allergy describes an inappropriate immune response to a non-pathogenic antigen. The term allergy is often used synonymously to describe atopic reactions. Allergy is a broader term including all mechanisms of hypersensitivity. However, as atopic reactions are extremely common, and other types of allergic reactions are rare, most allergic reactions are atopic.

Production of IgE frequently leads to Type I hypersensitivity reactions. Clinically this type of immune response is associated with asthma, eczema, rhinitis, urticaria, angioedema and anaphylaxis.

PHASE I – SENSITISATION On initial exposure to allergen, no clinical manifestations occur. Antigen is presented to the immune system and TH2 cells are produced. TH2 cells promote production of IgE, rather than other types of antibody. IgE binds to high-affinity IgE receptor on mast cells. Mast cells contain granules packed with histamine, tryptase and other inflammatory mediators and when stimulated produce leukotrienes and a number of cytokines.

PHASE II - EXPOSURE AND EARLY PHASE Once mast cells are coated with allergen-specific IgE, reactions may occur on subsequent allergen exposure. Cross-linking of surface-bound IgE activates the mast cell, leading to rapid degranulation (Figure 9.2). Histamine and leukotrienes are prominent early mediators and cause vasodilatation, increased vascular permeability, itch and bronchocon- striction. Vasodilatation causes swelling of tissues, erythema of skin and if generalised, hypotension. Increased vascular permeability in the skin causes wheals seen in an urticarial reaction, and angioedema when subcutaneous tissues are involved. In the airway, mucosal oedema contributes to airway narrowing in acute asthma.

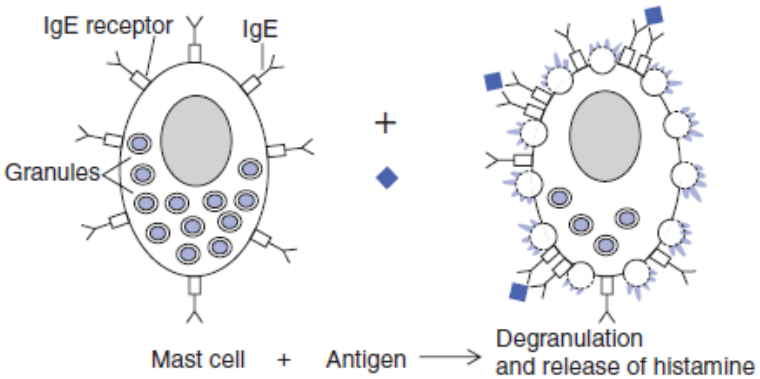

Figure 9.2 Antigen cross-linking of surface bound IgE on mast cell causes degranulation and release of histamine.

PHASE III - LATE PHASE OF ATOPIC INFLAMMATION Leukotrienes and cytokines produced by the mast cells attract monocytes, eosinophils and T cells to the site of inflammation. By 6-12 hours a mixed inflammatory response with abundant eosinophils is seen at the site of allergen exposure. Eosinophil products prolong the inflammatory response. The IL-4 rich environment predisposes to further TH2 cell development and subsequent IgE production. After repetitive exposure to an allergen, the inflammatory late phase of the response may become chronic. Once this occurs, histamine plays a minor role, with eosinophils playing a major role in tissue injury (Figure 9.3). Tissue remodelling may occur, leading to irreversible changes.

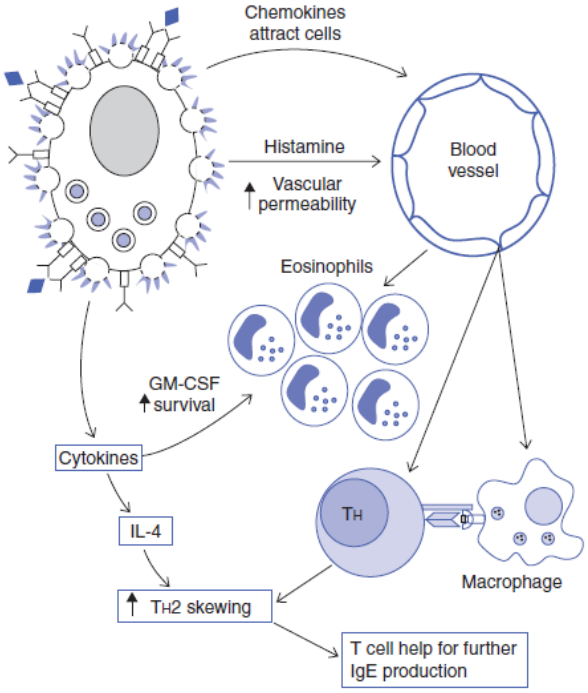

Figure 9.3 Late phase of atopic inflammation is characterised by eosinophil dominated inflammation.

Clinically, the late phase of the inflammatory response causes recurrence of symptoms, often with a severity comparable to the initial symptoms. Thus a patient who responds to treatment initially for an acute asthmatic attack may deteriorate significantly a few hours later, if appropriate treatment is not given.

CLINICAL MANIFESTATIONS OF ATOPY Atopy is a genetic predisposition to produce IgE in response to antigen. An IgE response to allergens is necessary, but not sufficient to produce clinical manifestations of atopy. Not everyone who is atopic develops atopic disease, as environmental influences are also important. Sensitisation to a single allergen can occur, but the majority of atopic individuals become sensitised to multiple allergens. Asthma, rhinitis and eczema comprise the so-called ‘atopic triad’.

In utero, the developing immune system is biased towards TH2 responses. In non-atopic individuals this rapidly switches to predominantly THl-type responses, while atopic children continue to develop TH2 responses for a prolonged or indefinite period. The reasons for this are poorly understood.

EPIDEMIOLOGY Atopy is common, and the incidence is rising. A family history is a significant risk factor for the development of atopy. Approximately 60% of children with two atopic parents and 40% of those with one atopic parent develop atopic disease. However, 10% of children in families where neither parent is atopic also develop atopic disease.

Environmental factors in early childhood appear to greatly influence whether atopic disease will develop. Children with older siblings, or attending nurseries appear less prone to developing atopic diseases, possibly due to increased exposure to infection early in life. It has been suggested that better hygiene with decreased infection rates contributes to the increased prevalence of allergy - the so-called 'hygiene hypothesis'. Other environmental factors thought to contribute include early exposure to allergens (house dust mite and pet dander as well as outdoor allergens), dietary allergens (particularly highly allergenic foods such as milk, egg, nuts and fish), and pollution including cigarette smoke.

Approximately 15% of children in Western countries suffer from asthma, 15% have rhinitis, and 10% have eczema. Epidemiological studies record a significantly increased prevalence of asthma over the last three decades.

CLINICAL MANIFESTATIONS The classic atopic triad consists of asthma, eczema and rhinitis, but atopic individuals also have an increased incidence of urticaria, angioedema and anaphylaxis. The following sections describe many atopic disorders; however, a brief outline of rhinitis and eczema is included in this section.

Rhinitis

Rhinitis means inflammation of the nasal airways. This may result in sneezing, itch, congestion, nasal blockage, disturbed smell and taste and occasionally nasal polyps. Allergic rhinitis is commonly accompanied by conjunctivitis. Rhinitis may be infective, allergic or non-allergic (Table 9.2).

The diagnosis of rhinitis is based on history. Clinical examination excludes nasal polyps or a deviated septum as the cause of obstruction. Skin prick tests (SPTs) or measurement of allergen-specific IgE to common aeroallergens is useful.

Treatment consists of:

♦Allergen avoidance, where possible.

♦Topical nasal steroids, using a spray or drops. Nasal steroid sprays are ineffective if the nasal airway is obstructed. However, once patency is restored using nose drops, sprays are a more convenient maintenance therapy.

♦Non-sedating antihistamine may be added if required.

Allergen immunotherapy appears to be effective for allergic rhinitis caused by mites, pollen or animal dander. It is very time-consuming for the patient and clinical staff, and may be reserved for when allergen avoidance is impossible and pharmacotherapy has failed.

When rhinitis fails to respond to appropriate therapy, the diagnosis should be reviewed, particularly when nasal obstruction is the prominent symptom. Nasopharyngeal tumours, Wegener's granulomatosus, nasal polyps, deviated septum, adenitis and the presence of a foreign body should be excluded.

Table 9.2 Rhinitis: causes and clinical features.

CAUSE	TYPES	COMMENTS
Infective	Viral Bacterial	Symptoms self-limiting, may be associated with sinusitis. Occasionally purulent discharge (e.g. 'common cold')
Allergic	Seasonal Perennial	Symptoms over summer months.Commonly pollen allergy Symptoms all year. Common allergens include house dust mite, cat and other pets, and occasionally moulds
Non-allergic	Rhinitis medicamentosa Eosinophilic Vasomotor Other	Chronic nasal obstruction due to overuse of sympathomimetic decongestants Chronic symptoms. Pathogenesis poorly understood. Usually respond to similar therapy as allergic rhinitis

Atopic eczema/dermatitis

Atopic eczema/dermatitis (AD) is a chronic inflammatory skin disorder affecting approximately 10% of children. There is considerable geographical variation in prevalence, with highest rates in Northern Europe and Australia. Approximately 40% of individuals with AD will have associated rhinitis and/or asthma, however, these atopic manifestations may appear later.

AD usually begins in early childhood, and the majority of children outgrow it, although a small number experience recurrence during adolescence (Figure 9.3). It is rare to see new onset AD in adults, and most adults who have AD have had the disease since childhood.

SPTs are positive in the majority of patients with AD, but allergens identified correlate poorly with symptoms. Allergen challenges have a better predictive value, and therefore putative allergens identified by SPT should be evaluated by food avoidance and challenge. Food allergy plays a role in approximately one third of AD patients, usually those with early onset of severe disease. The majority of positive food challenges involve egg, milk, wheat, soya and peanut. In adults, contact sensitivity may play a role in some patients with AD, and patch tests may be useful in assessing such allergens. Children with AD are at increased risk of infection, which may lead to an exacerbation of inflammation.

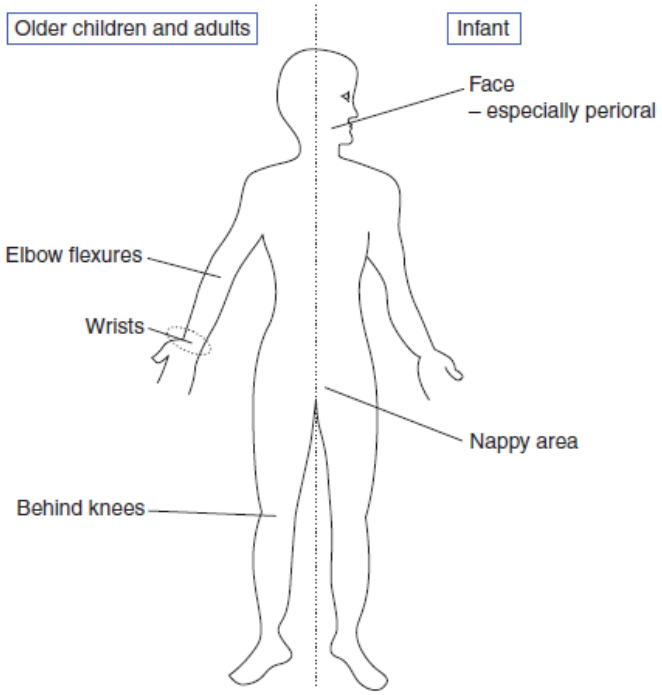

Figure 9.3 Typical distributions of eczema in adults and infants. Severe eczema may be generalised at any age.

Treatment of AD includes:

♦Avoidance of allergens and irritants

♦Emollients to ensure skin is constantly well moisturised

♦Topical steroids for exacerbations

♦Antibiotics for infection

♦More potent topical and systemic immunomodulatory therapies are available for severe disease.

ASTHMA

Asthma is a lung disease, characterised by reversible airway obstruction, airway inflammation and airway hyper-responsiveness to allergens and irritants. Asthma is frequently due to atopy, however, other causes account for 20-30% of cases (Table 9.3).

Table 9.3 Types of asthma

TYPE OF ASTHMA	FEATURES
Atopic	Associated with atopic disorders. High serum IgE and sensitisation to common aeroallergens
Occupational asthma	Sensitisation to specific allergen, associated with work/hobby. Symptoms initially exposure-related, may become chronic
Aspirin-sensitive asthma	Asthma often severe and brittle. Associated with rhinitis and nasal polyps. Responds well to desensitisation
Intrinsic	Mechanism poorly understood. Early or late onset. Frequent eosinophilia and sinusitis. No allergen sensitisation
Asthmatic component of other lung disease	Chronic obstructive pulmonary disease, bronchiectasis
Associated with vasculitis	Churg–Strauss syndrome – late onset asthma. Marked eosinophilia. Precedes vasculitis by years

PATHOGENESIS Genetic factors play a role in determining both atopic tendency and predisposition to asthma. However, environmental factors also play a significant role.

Airways obstruction in asthma is due to:

- ♦Contraction of airway smooth muscle
- ♦Inflammation of airway mucosa
- ♦Hypersecretion of mucus.

Inflammatory mediators (histamine, leukotrienes, bradykinin and prostaglandins) and neural influences cause contraction of airway smooth muscle. Airway inflammation follows with eosinophil and TH2 lymphocyte infiltration. Inflammation increases the ratio of mucus-producing cells to ciliated cells in the airway. Increased mucus production coupled with decreased mucus clearance by ciliated cells causes accumulation of mucus in the airway, which may become plugged during severe attacks. Chronic bronchospasm eventually leads to hypertrophy of bronchial smooth muscle. Chronic inflammation leads to airway remodelling with thickening of the basement membrane and fibrosis. Long-term control of asthma is critically dependent on controlling airway mucosal inflammation (Figures 9.4 and 9.5).

EPIDEMIOLOGY The prevalence of asthma is rising, with approximately 15% of children and 5-10% of adults affected. While awareness is increased, it is generally accepted that the true prevalence is rising. Prevalence has increased within one generation, which cannot be explained by genetic factors, implying a significant environmental component. Recent studies in Leipzig have shown that Westernisation of lifestyle is associated with a significant increase in childhood asthma.

CLINICAL PRESENTATIONS Asthma classically presents with episodic wheeze, breathlessness and cough. Symptoms usually show diurnal variation with ‘morning tightness’ - maximal wheeze and cough early in the morning, improving as the day goes on.

Mild asthmatics may be apparently symptom-free for days or weeks. Generally, however, there is mild airway inflammation even when symptom-free, with further inflammation triggered by infection or increased allergen exposure. In more severe disease symptoms are present most days, but vary in intensity.

In ‘cough variant’ asthma episodic or chronic dry cough is the only complaint. Exercise commonly precipitates wheeze or cough as the associated cooling and drying irritates the airway mucosa. However, particularly in children, exercise-induced wheeze may be the only symptom.

DIAGNOSIS Asthma should be considered in patients with a history of cough and wheeze. Physical examination may be normal or wheeze may be audible on auscultation. Spirometry

demonstrating airflow obstruction with >15% reversibility is diagnostic. Peak flow monitoring showing >20% variability is also suggestive of asthma.

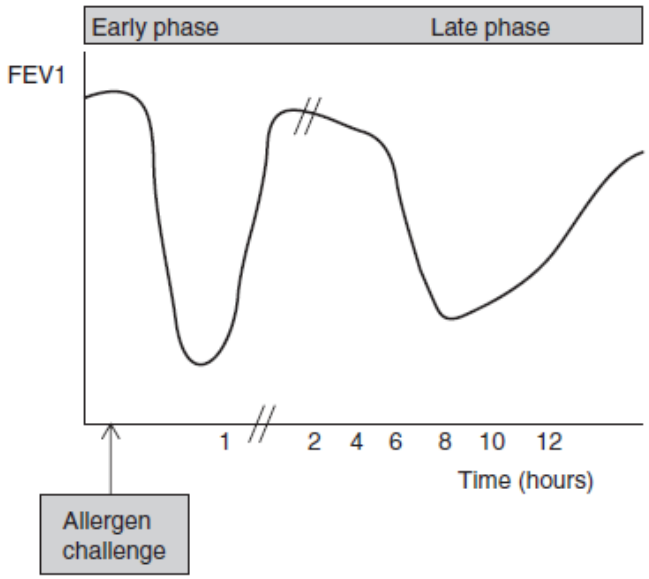

Figure 9.4 Early and late phase of the allergic response to allergen challenge.

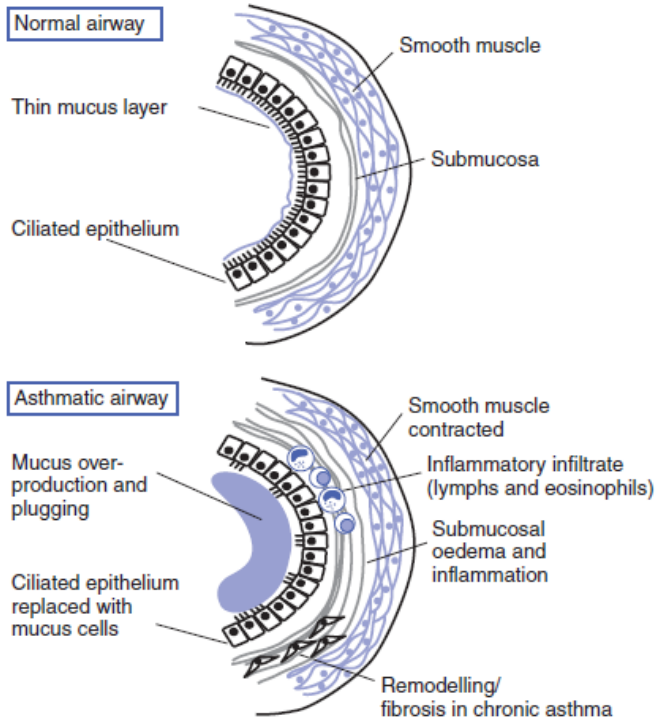

Figure 9.5 Changes in the asthmatic airway. Inhaled steroids are required to reverse inflammation.

If no variability is evident, bronchial provocation testing with histamine may demonstrate airway hyperreactivity. When exercise-induced asthma is suspected, comparing peak flows before and after 6-10 minutes of running may establish the diagnosis.

Measurement of IgE as well as SPT to determine specific allergies is of value.

TREATMENT OF ASTHMA Treatment of asthma is aimed at:

♦Controlling airway inflammation

♦Alleviating symptoms.

When specific allergens are identified, allergen avoidance may make asthma easier to control. However, allergen avoidance is demanding for the patient and often expensive to maintain. Unfortunately partial allergen avoidance is rarely beneficial.

Short-acting β2 agonists relax airway smooth muscle and relieve symptoms. They may be used alone to treat mild intermittent asthma. Mild persistent asthma is usually treated with regular inhaled steroids, with a short-acting β2 agonist as required. As disease becomes more severe prophylactic treatment is stepped up with:

♦High dose inhaled steroids

♦Long-acting β2 agonists

♦Leukotriene antagonists.

In severe asthma, sustained release theophylline and even oral steroids may be required to control airway inflammation. Short-acting β2 agonists and also anti-cholinergic drugs are added as required to control symptoms.

MANAGEMENT OF ACUTE ALLERGIC REACTIONS Acute allergic reactions cause symptoms varying from mild skin rash to life-threatening anaphylaxis. Accurate identification of the responsible allergen(s) often allows successful allergen avoidance. However, identification of unusual allergens may be impossible, particularly when reactions are infrequent and details about reactions are vague. This part describes the emergency management of an acute allergic reaction, as well as the long-term management of affected patients.

EMERGENCY MANAGEMENT OF AN ACUTE ALLERGIC REACTION Emergency management of an acute allergic reaction is summarised in Figure 9.6.

The drugs used for resuscitation are primarily adrenaline, fast-acting antihistamines and corticosteroids.

Adrenaline is a physiological antagonist of many of histamine effects. Adrenaline reverses bronchospasm, reduces angioedema and increases blood pressure by reversing vasodilation and increasing cardiac output. Adrenaline is given intramuscularly in doses of 0.5-1 mg (0.01 mg/kg). This dose can be repeated every 15 minutes until a response is obtained. In patients taking non-cardioselective β-blockers, addition of IV salbutamol should be considered.

A fast-acting antihistamine such as chlorpheniramine given by slow IV injection minimises further effects of histamine. In serious reactions adrenaline should be given IM immediately, and then IV access can be secured to give other agents.

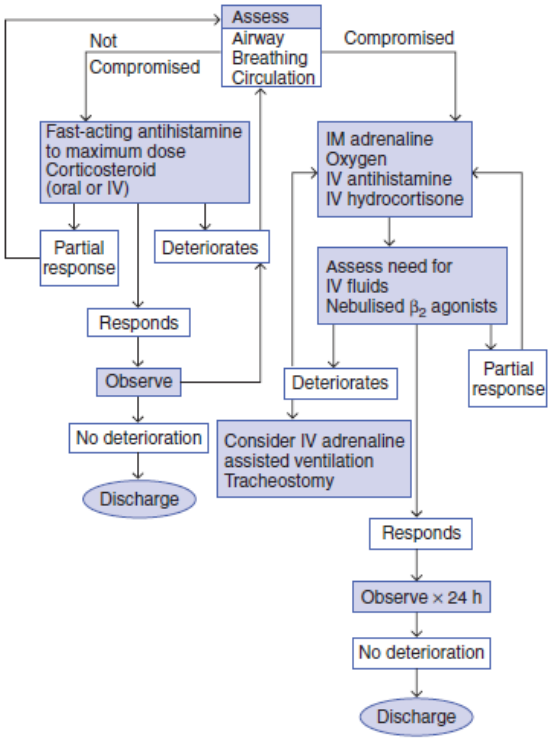

Figure 9.6 Emergency management of an acute allergic reaction.

Corticosteroids, usually IV hydrocortisone, take several hours to act. Steroids prevent or reduce secondary late phase reactions. Administration of rapidly acting medications obviously takes priority.

Where there is any respiratory difficulty oxygen should be administered. Intravenous fluids and nebulised β2-agonists are frequently required.

Patients who have a reaction requiring the use of adrenaline should be monitored for 8-24 hours in hospital as late phase reactions may cause deterioration. When discharged, patients should be given advice about further reactions and a prescription for a non-sedating fast-acting antihistamine to take at the onset of reactions. Short-term steroid treatment is particularly useful where there is an asthmatic component to the reaction. Arrangements should also be made for a specialist allergy opinion.

NON-ACUTE ASPECTS OF MANAGEMENT When patients have experienced an acute allergic reaction, further management aims to prevent recurrence and minimise the risks of future reactions.

Essential aspects of management of include:

♦Identification of the allergen(s) responsible.

♦Patient education about allergen avoidance.

♦Treatment of associated asthma if present.

♦Avoidance of drugs which exacerbate reactions or hinder resuscitation (especially β-blockers).

♦Formulation of an individualised emergency plan, with appropriate patient education to ensure that patients can manage future reactions appropriately.

♦Ensure allergies to medication are documented in a manner accessible to all healthcare providers, to prevent future avoidable reactions (e.g. MedicAlert bracelet, case note labelling).

Investigations to identify the allergen(s) responsible are helpful to take a detailed history of foods and medications recently ingested as soon as possible. Full investigation may take some time, and patients should have an emergency plan while investigations are proceeding, as the risk of further reactions is highest before the allergen(s) are identified.

Underlying asthma is frequently exacerbated during an allergic reaction. Regular use of short-acting β2 agonists is associated with reduced β2 receptor expression in the lung, limiting the effectiveness of adrenaline and salbutamol in reversing bronchospasm, should resuscitation be required. Adequate use of inhaled steroids and other preventive medication to avoid overuse of short-acting β2 agonists is essential.

Concomitant medications may exacerbate reactions or make resuscitation difficult. β-blockers inhibit many of the key actions of adrenaline (reversing bronchospasm and increasing cardiac output). Use of adrenaline in patients taking β-blockers and tricyclic antidepressants may be associated with severe hypertension. Angiotensin converting enzyme (ACE) inhibitors can cause angioedema, and may exacerbate allergic reactions. The risk-benefit ratio of any of the above medications should be carefully reviewed in patients who have had severe allergic reactions or where inadvertent allergen exposure is a significant risk, for example, in nut allergy. Patients should also be warned that alcohol and non-steroidal anti-inflammatory drugs (NSAIDs) may increase the severity of reactions.

Even the most conscientious patient is likely to ingest foods inadvertently, and therefore it is essential that patients understand their own emergency plan. Mild reactions may be treated with a fast-acting, non-sedating antihistamine. Patients with asthma should also use high-dose, short-acting β2 agonist immediately. More severe reactions including laryngeal oedema, bronchospasm or symptoms of hypotension require rapid medical attention. Self-administration of adrenaline while waiting for an ambulance may be life-saving. Adrenaline in a dose suitable for self-administration (300 gg) is available in pre-filled, ready-to-use syringes.

All allergies to medications should be documented and the information transmitted to other healthcare providers. Patients with allergy to a medication commonly used in an emergency, or to latex should wear a MedicAlert bracelet.

Desensitisation is a procedure that aims to prevent allergic reactions by exposure to minute doses of allergen, which are gradually increased. Not surprisingly, this form of therapy carries a significant risk of anaphylaxis. It is contraindicated in patients with severe asthma, significant ischaemic heart disease or those who cannot discontinue β-blocker therapy. Desensitisation is used for patients who have had systemic reactions to wasp and bee venom, and occasionally for drug allergies. Desensitisation may also be used in patients with allergies to inhaled antigens, unless there is associated asthma. Unfortunately, there is no safe and effective form of desensitisation available for food allergies.

ANAPHYLAXIS

Anaphylaxis is a severe allergic reaction that includes at least one life-threatening feature - hypotension, bronchospasm or laryngeal oedema. Anaphylactic shock means hypotension due to allergy, usually occurring with other allergic features. Anaphylaxis is due to IgE mediated mast cell activation. Clinically indistinguishable reactions occur when mast cells are triggered by mechanisms other than IgE cross-linking - these are called anaphylactoid reactions.

WHAT CAUSES ANAPHYLACTIC REACTIONS? Anaphylaxis can result from allergen entering the body by any route. In extreme cases, smelling or touching the offending agent may trigger a reaction. Common precipitants include:

- ♦Drugs - penicillins, cephalosporins, anaesthetic agents including local anaesthetics
- ♦Foods - especially peanuts, tree nuts, fish, shellfish, egg, milk and soya
- ♦Venoms - including bee and wasp
- ♦Latex.

Mechanisms involved in anaphylactoid reactions include:

- ♦Complement activation - immunoglobulin and colloid infusions
- ♦Hypertonic solutions activating mast cells - X-ray contrast media
- ♦Unknown - non-steroidal anti-inflammatory agents.

WHAT IMMUNOLOGICAL MECHANISMS ARE INVOLVED? The final common pathway of anaphylactic and anaphylactoid reactions is mast cell activation. Release of histamine and other inflammatory mediators (including tryptase, chymase, heparin) causes the early phase of allergic reactions. Once triggered, mast cells synthesise prostaglandins, leukotrienes and cytokines involved in late phase responses. Because the immediate response is due to release of preformed mediators, the onset of anaphylaxis is within minutes of exposure to the allergen.

CLINICAL FEATURES Anaphylaxis frequently affects many organ systems. Prominent symptoms and signs include:

- ♦Skin - flushing (due to vasodilation), itch, urticaria and angioedema
- ♦Upper airways - sneezing and laryngeal oedema
- ♦Lung - bronchospasm (contraction of bronchial smooth muscle, mucus production and oedema of airway mucosa)
- ♦Heart - tachycardia and arrhythmias
- ♦Cardiovascular - hypotension (vasodilation and increased vascular permeability)
- ♦Gut - nausea, vomiting, cramps, diarrhoea (spasm of GI muscle)
- ♦Nervous system - anxiety, dizziness, loss of consciousness.

INVESTIGATIONS A full and accurate history of all foods/drugs and exposures prior to the reaction is essential. Exposure is usually within a few hours and often minutes of the reaction. It is also important to identify any exacerbating factors that may have been present including:

- ♦Infection
- ♦Exercise
- ♦Alcohol

♦Non-steroidal agents

♦β-blockers and ACE inhibitors.

Investigations are divided into:

♦Tests differentiating anaphylaxis from other disorders

— Mast cell tryptase

— Urinary methylhistamines.

♦Tests to identify the allergen responsible

— SPTs or intradermal skin testing

— Allergen specific IgE.

Full investigation of the cause of the reaction is not undertaken until the patient has recovered. If history and straightforward investigations fail to identify the cause, provocation testing may be.

TREATMENT Immediate treatment requires adrenaline as well as antihistamines and corticosteroids. Long-term management of patients includes education about allergen avoidance and emergency self-administration of adrenaline. It is also essential to ensure that asthma is maintained under good control, as poorly controlled asthma is a major risk factor for mortality in future attacks.

Desensitisation to the allergen is possible in some situations (e.g. venom allergy) where avoidance of allergen is not possible and appropriate preparations are available. Desensitising protocols have been described for some drugs, but these procedures are labour-intensive and carry a risk of provoking anaphylaxis. Hence, desensitisation is usually reserved for patients where there is no alternative therapy available.

THEME №10. OTHER ALLERGIC (NOT ATOPIC) DISEASES

DRUG ALLERGY

DEFINITION OF DRUG ALLERGY — A drug allergy, or an allergic drug reaction, is an adverse drug reaction that results from a specific immunologic response to a medication. The term immunologic drug reaction is also used to describe these reactions.

The classification and clinical features of drug allergy will be reviewed here, beginning with a categorization of the different types of adverse drug reactions. The pathogenesis of drug allergy is discussed elsewhere.

ADVERSE DRUG REACTIONS — An adverse drug reaction is a general term referring to any untoward reaction to a medication. Adverse drug reactions may be broadly divided into two types (table 10. 1):

Type A reactions — Type A reactions can affect any individual, given sufficient dose and exposure, and are predictable from the known pharmacologic properties of a drug. These make up 85 to 90 percent of all adverse drug reactions. Examples of type A reactions include diarrhea in response to antibiotics, gastritis in association with nonsteroidal antiinflammatory drugs, or aminoglycoside nephrotoxicity.

Type B reactions — Type B reactions make up 10 to 15 percent of adverse drug reactions. These are hypersensitivity reactions that occur in a susceptible subgroup of patients, have signs and symptoms that are different from the pharmacologic actions of the drug, and usually cannot be predicted.

Notable exceptions are the **predictable** hypersensitivity reactions with abacavir, carbamazepine , allopurinol, and flucloxacillin in patients with certain HLA types. HLA testing of patients is recommended before administering abacavir and carbamazepine in populations in which there is appreciable prevalence of the genotype. Specific screening recommendations are discussed in more detail separately.

Type B reactions may be further subdivided as follows:

Table 10. 1 Classification of adverse drug reactions

Drug reaction	Examples
Reactions occurring in most normal patients, given sufficient dose and duration of therapy (type A)	
Overdose	Hepatic failure (acetaminophen)
Side effects	Nausea, headache (with methylxanthines)
Secondary or indirect effects	GI bacterial alteration after antibiotics
Drug interactions	Erythromycin increasing theophylline/digoxin blood levels
Drug hypersensitivity reactions restricted to a small subset of the general population (type B)	
Intolerance*	Tinnitus after a single aspirin tablet
Idiosyncrasy • (pharmacogenetics)	G6PD deficiency: anemia after antioxidant drugs
Immunologic drug reactions (allergy)	Anaphylaxis from beta-lactam antibiotics

GI: gastrointestinal; G6PD: glucose-6-phosphate dehydrogenase.
* Side effects at subtherapeutic doses.
• Drug effect not attributable to known pharmacologic properties of drug and not immune medicated.

Exaggerated sensitivity to known drug toxicities — Some patients experience the pharmacologically predictable toxicity of a drug (or several drugs) at low and sometimes subtherapeutic doses. An example would be the individual who develops tinnitus in response to a single dose of aspirin. This putatively reflects altered drug metabolism or increased end-organ sensitivity. Patients with this type of drug intolerance are usually identifiable by a careful review of past adverse drug reactions.

Idiosyncratic drug reactions — Idiosyncratic drug reactions are qualitatively distinct from the known pharmacologic toxicities of the drug. These reactions can arise from genetic differences in the patient, such as primaquine causing nonimmune hemolytic anemia in patients with glucose-6-phosphate dehydrogenase deficiency.

Aside from a few well-described examples, the mechanisms of most idiosyncratic drug reactions remain obscure and probably reflect complex interactions of metabolic and constitutional factors.

Immunologic drug reactions (drug allergy) — Drug allergies result from specific immunologic responses to medications. Allergic drug reactions account for about 6 to 10 percent of all adverse drug reactions, but up to 10 percent of fatal reactions.

CLASSIFICATION OF DRUG ALLERGY — There are different systems of classifying immunologic drug reactions.

Classification based upon timing of symptom onset — The World Allergy Organization (WAO) has recommended dividing immunologic drug reactions into immediate reactions (ie, onset within one hour of exposure) and delayed reactions (onset after one hour), based upon the timing of the appearance of symptoms.

Immediate — The WAO distinction between immediate and delayed drug reactions is intended to distinguish IgE-mediated, type I reactions from other types. Type I reactions classically begin within one hour of the first administered dose. However, some IgE-mediated reactions appear after one hour, particularly after oral administration of a drug. In addition, taking the medication with food further slows absorption. Nevertheless, this period of one hour identifies the majority of IgE-mediated reactions, which carry the risk of anaphylaxis if the patient is re-exposed.

Delayed — Reactions appearing after one hour are classified as delayed, although most delayed reactions begin after six hours and typically after days of treatment. As an example, delayed reactions to amoxicillin classically start on day 7 to 10 of treatment, and may even begin 1 to 3 days after cessation of treatment. These reactions may be caused by several different mechanisms, but are not IgE-mediated.

Some delayed reactions begin after weeks of continuous treatment. One such disorder is "drug rash with eosinophilia and systemic symptoms" (DRESS), which is a systemic drug reaction that begins 1 to 12 weeks into continuous treatment. This reaction, which is also called "drug-induced hypersensitivity syndrome" (DiHS), is characterized by fever, rash, and multiorgan involvement. The liver (hepatitis) and heart (hypersensitivity myocarditis) may be affected. These reactions can persist for weeks to months, even after the medication is stopped.

TYPE I REACTIONS — Type I reactions require the presence of drug-specific immunoglobulin E (IgE). Some patients form drug-specific IgE upon exposure to a medication, although most do not.

Once formed, drug-specific IgE occupies surface receptors on mast cells and basophils throughout the body. If the drug is encountered again, it may bind to these IgE molecules, causing crosslinking of the receptors and activation of the cells, resulting in symptoms. IgE-mediated reactions are dose-dependent, although this may not be clinically apparent because even very low doses can cause severe systemic symptoms.

Clinical features — The signs and symptoms of type I reactions are directly attributable to the vasoactive mediators released by mast cells and basophils. The most common signs and symptoms are urticarial rash; pruritus; flushing; angioedema of the face, extremities, or laryngeal tissues (leading to throat tightness with stridor, or rarely asphyxiation); wheezing; gastrointestinal symptoms; and/or hypotension.

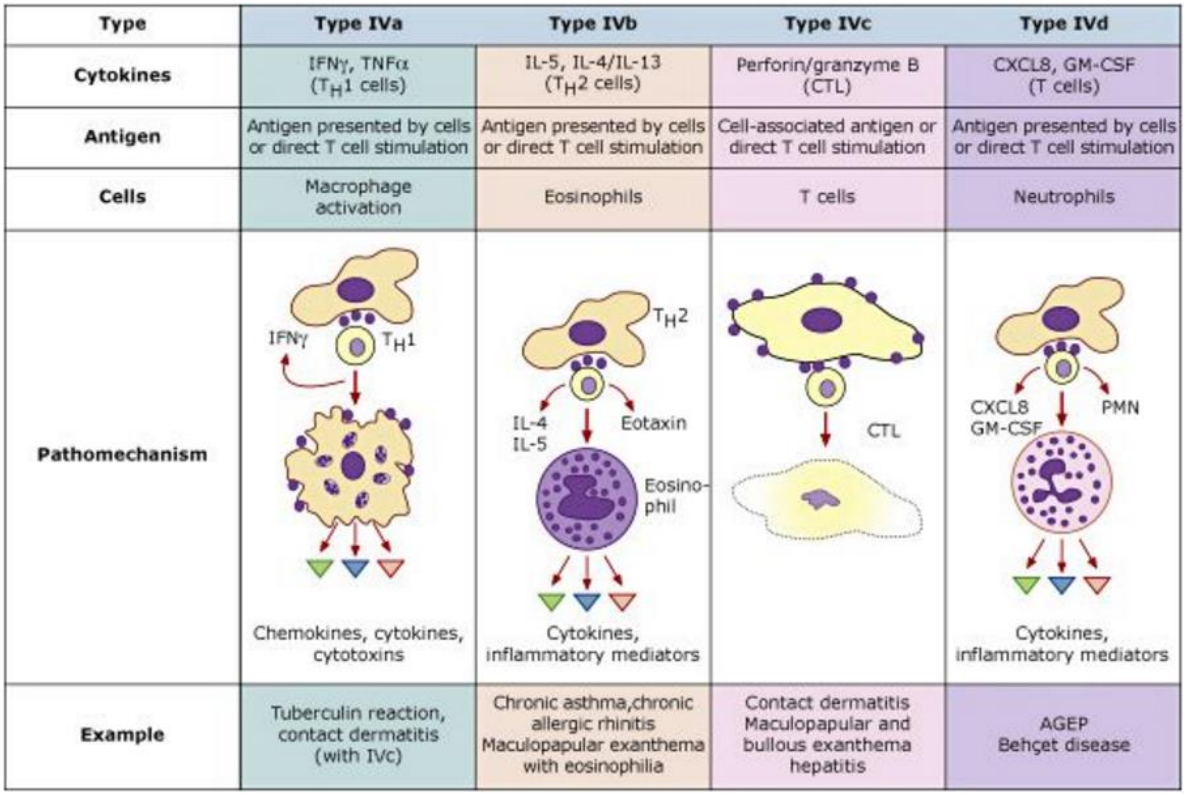

Figure 10. 1. T cell mediated hypersensitivity reactions (Gell and Coombs types IVa-d)

Anaphylaxis is the most severe presentation of an IgE-mediated drug reaction. Mast cell tryptase and histamine can be elevated in the circulation just after anaphylaxis, and the detection of these mediators implicates mast cells and basophils in the reaction, supporting the diagnosis of anaphylaxis.

Neither fever nor elevations in serum C-reactive protein are seen with IgE-mediated reactions. The absence of these features can help distinguish IgE-mediated reactions from some other adverse drug reactions.

The presence of urticaria is useful in identifying IgE-mediated reactions, because the classical wheal and flare are hallmark signs of mast cell degranulation. However, other skin findings in drug reactions can mimic urticaria and it can be difficult to discern if a rash was truly urticarial based upon history alone. Many delayed reactions involve a pruritic exanthem or rash that causes diffuse swelling of the skin, and affected patients will report raised, itchy areas of skin. However, these delayed-onset edematous exanthems are NOT urticarial rashes. In addition, they are generally not dangerous, provided the skin does not blister or slough and there are no signs of organ inflammation. Reactions involving rash and organ inflammation are discussed below.

Timing — The timing of onset of type I reactions is rapid but varies with the clinical setting and presentation:

- IgE-mediated reactions occur rapidly after the last administered dose, which underlies the designation of immediate by the World Allergy Organization. The time to onset is influenced by the route of administration: intravenously administered medications may cause symptoms in seconds to minutes, while the same drug administered orally may cause symptoms in 3 to 30 minutes if taken on an empty stomach, and in 10 to 60 minutes if taken with food.
- IgE-mediated anaphylactic reactions should NOT begin several days into a course of therapy, if the patient's exposure to the drug has been continuous. However, if several doses are skipped, symptoms can appear when the drug is resumed.
- Isolated urticarial rashes can occur late during continuous therapy. These rashes are relatively common and sometimes look like an intermediate between an urticarial and maculopapular eruption. The delayed appearance of urticarial rashes with known allergenic drugs like beta lactam antibiotics may reflect time required for significant IgE responses, just as for late-occurring serum sickness reactions. However, late urticarial rashes with drugs that rarely cause acute allergy (eg, macrolide antibiotics) makes it unlikely that many of these reactions are IgE-mediated and T cells may also be involved, although the pathogenesis is not known. Tests for immediate-type allergy are usually negative.
- Rash and urticaria appearing within minutes to hours after drug intake can also be seen in "pseudoallergic" reactions.

Commonly-implicated drugs — The drugs most commonly implicated in type I reactions include the following:

- Beta lactam drugs (penicillins and cephalosporins).
- Neuromuscular blocking agents.
- Quinolones: Quinolone antibiotics have been implicated as a common cause of hypersensitivity reactions in Europe but less commonly in the United States, so there may be important geographical variations that will become more apparent over time. Only a subset of these reactions is IgE-mediated.
- Platinum containing chemotherapeutic agents, such as carboplatin and oxaliplatin .
- Foreign proteins, including chimeric antibodies such as cetuximab and rituximab : The frequency with which these agents cause anaphylaxis can also vary by geographical location (eg, cetuximab-induced anaphylaxis has been reported mostly in the Southern United States).

Previous exposure to the suspect drug — IgE-mediated reactions generally require previous exposure to the drug in question. However, the absence of a known prior exposure does not exclude an IgE-mediated reaction, because sensitization may have occurred from exposure to a cross-reactive compound, even though the patient showed no signs of allergy to the sensitizing product.

The following examples illustrate this phenomenon:

- A significant percentage of patients experiencing anaphylaxis upon first exposure to neuromuscular blocking agents are believed to have been previously sensitized through the use of various cosmetics, personal products, and nonprescription cough remedies (eg, pholcodine in Norway) that contain tertiary and quaternary ammonium groups. The ammonium groups shared by all of these agents are highly immunoreactive and can induce cross-reactive IgE antibodies.
- Some patients develop anaphylaxis to cetuximab and appear to have been previously sensitized to oligosaccharides present on the drug. The same oligosaccharides are found on several nonprimate mammalian proteins, such as beef, pork, and lamb, although the source of the original sensitization is still uncertain.

TYPE II REACTIONS — Type II reactions are uncommon and involve antibody-mediated cell destruction. Type II reactions may arise when drugs bind to surfaces of certain cell types and act as antigens. Subsequent binding of antibodies to the cell surface results in the cells being targeted for clearance by macrophages. Type II reactions may involve complement activation, but this is variable.

Clinical manifestations require the presence of high titers of preformed drug-specific IgG (or rarely IgM) antibodies, which are only made by a small percentage of individuals and usually in the setting of high-dose, long-term, or recurrent drug exposure. The factors predisposing individuals to form these antibodies are not fully understood.

Clinical features and timing — Type II drug reactions usually present as hemolytic anemia, thrombocytopenia, or neutropenia, since these are the cell types that are most often affected.

The clinical presentation can vary widely in severity; patients may be asymptomatic or present with fulminant illness. Symptoms usually appear at least five to eight days after exposure, but may begin after much longer periods of treatment. Symptoms can start within hours if the causative drug is stopped and then restarted.

Specific manifestations depend upon the cell type involved:

- Hemolytic anemia may present with dyspnea, varying degrees of fatigue, pallor, jaundice, dark urine, or signs and symptoms of the hyperdynamic state, such as bounding pulses, palpitations, and "roaring in the ears."
- The drugs most commonly implicated in hemolytic anemia are cephalosporins, penicillins, nonsteroidal antiinflammatory drugs (NSAIDs), and quinine / quinidine . The evaluation and diagnosis of drug-induced hemolytic anemia are discussed in greater detail separately.
- Drug-induced thrombocytopenia typically presents with petechial bleeding in the skin and buccal mucosa and isolated thrombocytopenia, often severe (ie, <20,000/microL) in a patient taking one or several medications. There may be splenomegaly and hepatomegaly due to platelet sequestration in these organs.
- Drugs implicated in thrombocytopenia include heparin , abciximab , quinine and quinidine , sulfonamides,vancomycin , gold compounds, beta-lactam antibiotics, carbamazepine , nonsteroidal antiinflammatory drugs, and others. This evaluation and diagnosis of this disorder are reviewed elsewhere.
- Severe neutropenia or agranulocytosis due to type II drug reactions presents days to weeks after beginning the medication, often with acute and clinically apparent symptoms of infection, such as fever, stomatitis, pharyngitis, pneumonia, or sepsis. Rechallenge or inadvertent subsequent administration is associated with a prompt recurrence, even with low doses.

Culprit drugs include propylthiouracil (PTU), the antimalarial drug amodiaquine and one of its major metabolites, mono-desethyl amodiaquine, and flecainide . The evaluation and diagnosis of this disorder are presented separately.

TYPE III REACTIONS — Type III reactions are mediated by antigen-antibody complexes. These reactions are uncommon and usually seen in the context of high-dose, prolonged drug administration, similar to type II reactions.

In a type III reaction, the drug acts as a soluble antigen and binds drug-specific IgG, forming small immune complexes that precipitate in various tissues, including blood vessels, joints, and renal glomeruli. These immune complexes bind to Fc-IgG receptors of inflammatory cells and/or activate complement, and an inflammatory response ensues. Reexposure to similar or higher doses of the same drug can cause a more rapid and severe recurrence.

Timing — Signs and symptoms take one or more weeks to develop after drug exposure, since significant quantities of antibody are needed to generate symptoms related to antigen-antibody complexes.

Clinical presentation — Type III reactions can take several forms:

Serum sickness — Classic serum sickness involves fever, urticarial or purpuric rash, arthralgias, and/or acute glomerulonephritis. Alternatively, just one or two of these features may be apparent. Other findings include lymphadenopathy, low serum complement levels, and an elevated erythrocyte sedimentation rate. The most common culprits are penicillin, amoxicillin , cefaclor , and trimethoprim-sulfamethoxazole .

Vasculitis — Drug-induced hypersensitivity vasculitis typically presents as palpable purpura and/or petechiae, fever, urticaria, arthralgias, lymphadenopathy, elevated erythrocyte sedimentation rate, and low complement levels. Purpuric lesions often affect the lower extremities. Uncommonly, other organs, such as the gastrointestinal tract or kidneys, are involved. The most common culprits are penicillins, cephalosporins, sulfonamides (including most loop and thiazide-type diuretics), phenytoin , and allopurinol .

Drug fever — Fever can be the sole symptom or the most prominent symptom of drug hypersensitivity, accompanied in a minority of cases by nonurticarial rash or other organ involvement. Medications implicated in causing drug fever include azathioprine, sulfasalazine , minocycline , trimethoprim-sulfamethoxazole , sirolimus , and tacrolimus . Drug fever can arise from type IV immunologic reactions as well.

TYPE IV REACTIONS — Type IV reactions are not mediated by antibodies, in contrast to the other three types above. Type IV drug reactions involve the activation and expansion of T cells, which requires time (normally many hours or days after antigen exposure), hence the name delayed type hypersensitivity (DTH). In some cases, other cell types (eg, macrophages, eosinophils, or neutrophils) are also involved.

Clinical presentations — Reactions involving T cells have prominent skin findings, because the skin is a repository for an enormous number of T cells. Many cutaneous T cells are primed memory-effector cells, which react rapidly if immunogenic agents penetrate the skin barrier or diffuse into the skin from the circulation.

Recognized patterns of cutaneous involvement include the following:

Contact dermatitis — Contact dermatitis is a reaction to topically applied drugs, which is characterized by erythema and edema with vesicles or bullae that often rupture, leaving a crust. Subacute and chronic contact dermatitis are characterized by lichenification, erythema, and scaling.

Morbilliform eruptions — Morbilliform or maculopapular eruptions are common and may arise from type IV immunologic reactions, as well as from other mechanisms.

Stevens-Johnson syndrome and toxic epidermal necrolysis — Severe exfoliative dermatitides, such as Stevens-Johnson syndrome and toxic epidermal necrolysis, are potentially life-threatening reactions characterized by fever and mucocutaneous lesions leading to necrosis and sloughing of the epidermis.

Drug-induced hypersensitivity syndrome — Drug-induced hypersensitivity syndrome (DiHS), also called drug rash with eosinophilia and systemic symptoms (DRESS), is a severe drug hypersensitivity reaction involving rash, fever (38 to 40°C) and multi organ failure. The liver, kidneys, heart, and/or lungs are most often affected. Drugs that have been implicated in causing DRESS/DiHS include several antiepileptics (including carbamazepine , phenytoin , and phenobarbital), minocycline , allopurinol , dapsone , abacavir and nevirapine .

Debate is ongoing about the most accurate name for this syndrome, as fewer than one-half of cases show peripheral eosinophilia (eg, those caused by abacavir or lamotrigine typically do not). The presence of atypical lymphocytes (CD8+) is a more consistent finding, which may persist for months after drug withdrawal.

Certain medications tend to cause inflammation predominantly affecting a specific organ. For example, anticonvulsant-induced DiHS frequently involves hepatitis, allopurinol can cause nephritis, and abacavir can cause pneumonitis.

Occasionally with T cell-mediated hypersensitivity, organ involvement occurs in the absence of skin findings. Presentations include isolated, drug-induced hepatitis, isolated interstitial nephritis, and isolated pneumonitis. Identification of this presentation as drug allergy can be challenging.

Some DiHS/DRESS reactions occur more frequently in patients with certain HLA types, a phenomenon that is also seen in Stevens-Johnson syndrome and toxic epidermal necrolysis. Specific examples include:

- DiHS/DRESS to allopurinol is associated with HLA-B*5801
- DiHS/DRESS, and in some cases SJS/TEN, to carbamazepine disproportionately affects several groups
- Han Chinese patients with HLA-B1502, as well as Thai, Malaysian, and Indian patients with this same allele
- Japanese and European patients with HLA-A*3101
- DiHS/DRESS to abacavir occur predominantly in patients with HLA-B*5701

Once a patient has been identified as having a high risk HLA profile, family members of that patient should also be advised to avoid the relevant drug, as familial occurrence of such hypersensitivity reactions has been noted. Recommendations have been made for screening patients for specific alleles prior to administration of carbamazepine , abacavir , andallopurinol .

Acute generalized exanthematous pustulosis — Acute generalized exanthematous pustulosis (AGEP) is a rare type of reaction characterized by superficial pustules, usually appearing within 24 hours after the administration of the culprit drug. This disorder is discussed in more detail separately.

Timing of type IV reactions — Type IV reactions are typically delayed in onset by at least 48 to 72 hours and sometimes by days to weeks following exposure to the culprit drug. Upon rechallenge, symptoms may appear within 24 hours. The time to symptom onset for reactions depends in part upon the number of T cells activated by the drug. These responses are polyclonal, and symptoms appear rapidly if the drug stimulates a large number of different T cell clones. In contrast, a drug that activates just a few clones may not cause clinical symptoms until these T cells have proliferated for several weeks.

Stevens-Johnson syndrome/toxic epidermal necrolysis (SJS/TEN) and DRESS/DiHS, the most dangerous of the delayed drug hypersensitivity reactions, often appear after weeks of uncomplicated treatment. Patients suddenly develop signs and symptoms of a fulminant immune reaction. This presentation results from uncontrolled expansion of oligoclonal T cells that have been massively stimulated by the drug, reminiscent of superantigen-like stimulation.

Association with viral infections — There is a higher risk of some type IV drug allergic reactions during generalized viral infections and exacerbations of autoimmune diseases, disorders in which T cell

reactivity is enhanced by widespread immune activation of T cells, high cytokine levels, and an increased expression of MHC- and costimulatory molecules.

Viral infections that predispose patients to reactions to certain drugs include the following:

- Epstein Barr virus (with amoxicillin).
- Cytomegalovirus (with antibiotics).
- Human herpes virus 6 (with anticonvulsants and other agents).
- Human immunodeficiency virus (HIV) infection (with trimethoprim sulfamethoxazole and other agents).
- In young children, treatment with amoxicillin (and to a lesser extent with other antibiotics) often elicits exanthematous reactions. Most of these children tolerate the same drug if given again later on. It is likely that systemic viral infections are facilitating these reactions as well, although this has not been conclusively demonstrated. Rhinoviruses, which cause only local infections of the nasopharynx and respiratory tract, should not have this effect because they do not stimulate the immune system to the same degree.

Association with dose — It is uncertain the extent to which drug dose (daily dose or duration of treatment) contribute to the development of delayed, T cell mediated drug allergies. Certain examples suggest that dose is important; more than 10 days of gemifloxacin caused maculopapular exanthema in nearly one-third of females, while this was rare with three days of treatment.

In allopurinol hypersensitivity, there are data to suggest that starting dose may be relevant for developing hypersensitivity.

Subcategories of type IV — T cells can orchestrate different forms of inflammation depending upon the cytokines produced and the other types of cells that become involved, leading to the subcategories of types IVa to IVd (figure 10. 1). These subcategories are discussed in detail separately.

OTHER TYPES OF REACTIONS

Drug-induced autoimmunity — Drugs can induce autoimmune diseases. Despite intensive scrutiny, the pathogenesis of these reactions has not been elucidated.

- The best known example is a lupus-like disease, which can develop after exposure to procainamide , phenytoin ,isoniazid , sulfasalazine , amiodarone , minocycline , and penicillamine .
- Penicillamine can also cause a pemphigus-like disorder.
- IgA bullous dermatosis has been associated with vancomycin and various other drugs, includingceftriaxone , ciprofloxacin , and metronidazole .

Fixed drug eruption — There are other drug-induced disorders that cannot be readily classified. As an example, fixed drug eruption is a relative common reaction, which is characterized by erythematous and edematous plaques with a grayish center or frank bullae. Lesions recur at exactly the same sites (typically lips and tongue, genitalia, face, and acral areas) with drug reexposure. These site(s) develop postinflammatory pigmentation. Fixed drug eruption can occur in response to sulfonamides, anticoagulants, and many other drugs. The mechanism is unknown, although T cells residing in the skin produce interferon gamma.

PSEUDOALLERGIC REACTIONS — Pseudoallergic drug reactions are adverse drug reactions with signs and symptoms that mimic immunologic drug allergies, but in which immunologic mechanisms have not been demonstrated. They are a subset of idiosyncratic reactions and are also called "non-immune hypersensitivity reactions".

Pseudoallergic reactions are difficult to distinguish clinically because they are similar or identical in presentation to true allergic reactions. The complete mechanisms underlying most of them are not known and may be different ` Some pseudoallergic reactions arise from direct (rather than immunologic) activation of immune and inflammatory cells, so the final steps in pathogenesis and the resultant clinical

features are indistinguishable from those of allergic reactions. However, the diagnosis, prognosis, and prevention are different. In particular, pseudoallergic reactions are not diagnosed with skin or in vitro allergy testing and do not worsen with repeated exposure.

Reactions that resemble anaphylaxis — Idiosyncratic reactions that mimic IgE-mediated, type I allergic reactions are among the most important for clinicians to understand. Like IgE-mediated reactions, nonimmunologic activation of mast cells and basophils results in the release of vasoactive mediators. These pseudoallergic reactions range in severity from mild to fatal. Accordingly, acute nonimmunologic anaphylaxis should be treated in the same manner as immunologic anaphylaxis.

Table 10. 3 Common examples of pseudoallergic drug reactions

Drug	Clinical reaction(s)	Presumed mechanism
Aspirin and other NSAIDs	Exacerbations of rhinitis, asthma (in patients with aspirin-exacerbated respiratory disease) Urticaria/angioedema (NOTE: urticaria may also result from a Type I, IgE-mediated allergic reaction)	Inhibited prostaglandin production and enhanced leukotriene production
Opiates	Pruritus, urticaria	Direct stimulation of mast cells and/or basophils causing release of mediators
Vancomycin	Flushing during infusion	Direct stimulation of mast cells and/or basophils causing release of mediators
Radiocontrast media	Anaphylaxis, shock (NOTE: some may be Type I, IgE-mediated allergic reactions)	unknown mechanism
Ciprofloxacin	Urticaria (most reactions)	Direct stimulation of mast cells and/or basophils causing release of mediators
Local anesthetics	Syncope	vaso-vagal reflex
Protamine	Hypotension, pulmonary hypertension	unknown mechanism
Choline	Pruritus, urticaria	unknown mechanism
Isoniazid	Hepatitis	unknown mechanism

NSAIDs: non-steriodal antiinflammatory drugs.

Nonimmunologic reactions resembling anaphylaxis are often referred to as "anaphylactoid." Unfortunately, the term anaphylactoid has been widely misinterpreted to mean a reaction that was similar to anaphylaxis but less severe, and has led to the undertreatment of patients with nonimmunologic anaphylaxis. Therefore, the term anaphylactoid is now discouraged, and the concept of nonimmunologic anaphylaxis is preferred. Clinicians should understand that all forms of anaphylaxis are potentially life-threatening and should treat affected patients appropriately.

It is unclear how certain drugs elicit these reactions. Some affected patients have underlying dermographism and seem to have an "instability" of their mast cells, which degranulate already upon pressure or by exposure to various small molecules.

Common culprit drugs — Drugs that can cause nonimmunologic anaphylaxis are listed below, and the clinical syndromes associated with each are reviewed separately:

- Radiocontrast agents
- Opiates
- Nonsteroidal antiinflammatory drugs (NSAIDs)
- Vancomycin
- Local anesthetic agents
- Chemotherapeutic agents
- Monoclonal antibodies and other biologic therapies used in cancer therapy

FOOD ALLERGY

An adverse food reaction is a general term for any untoward response to the ingestion of a food. Adverse food reactions can be divided into food allergies, which are immunologically mediated, and all other reactions, which are nonimmunologic (table 10. 4).

Table 10. 4. Nonimmunologic adverse food reactions

Gastrointestinal disorders	Intolerances
• Structural abnormalities • Hiatal hernia • Pyloric stenosis • Tracheoesophageal fistula • Hirschsprung's disease • Carbohydrate malabsorption • Lactase deficiency • Sucrose-isomaltase deficiency • Pancreatic insufficiency (cystic fibrosis) • Gastroesophageal reflux • Peptic ulcer disease • Gallbladder disease	• Pharmacologic agents • Caffeine • Theobromine (tea, chocolate) • Histamine-like compounds (fish, wine, sauerkraut) • Tryptamine (tomato, plum) • Tyramine (aged cheeses, pickled fish) • Serotonin (banana, tomato) • Phenylethylamine (chocolate) • Glycosidal alkaloid solanine (potatoes) • Alcohol • Flavorings and preservatives • Sodium metabisulfite • Monosodium glutamate
Toxic reactions	**Neurologic reactions**
• Seafood • Scromboid poisoning (fresh tuna and mackerel) • Ciguatera poisoning (grouper, snapper) • Saxitoxin (shellfish) • Other food poisoning • Clostridium botulinum • Staphylococcus aureus • Fungal toxins (aflatoxins, trichothecanes, ergot)	• Auriculotemporal syndrome
Psychologic reactions	**Accidental contaminations**
• Food phobias • Food aversions	• Pesticides • Antibiotics (if allergy present)

Adverse food reactions are common and often assumed by patients to be allergic in nature. However, nonimmunologic reactions to food are more common than true food allergies.

Food allergy is due to an abnormal immunologic response following exposure (usually ingestion) to a food. The terms allergy and hypersensitivity are used interchangeably in this topic review. There are multiple types of food allergy, each with distinct clinical and pathophysiologic features. Food allergies are

broadly categorized into either IgE-mediated or non-IgE mediated processes. Some disorders, such as atopic dermatitis or the eosinophilic gastrointestinal disorders, have characteristics of both mechanisms.

This topic reviews the clinical manifestations of the different categories of food allergies. Other aspects of food allergy are discussed separately.

IgE-MEDIATED REACTIONS — IgE-mediated food allergic reactions are rapid in onset, typically beginning within minutes to two hours from the time of ingestion. IgE-mediated reactions to carbohydrate allergens in meats, a type of reaction reported mainly in adults, represent an exception to this temporal pattern, since these reactions begin four to six hours after ingestion.

Signs and symptoms can involve the skin, respiratory and gastrointestinal tracts, and cardiovascular system and are believed to be caused by mediator release from tissue mast cells and circulating basophils (table **10. 5**). Two distinct presentations are the oral allergy syndrome and food-dependent, exercise-induced anaphylaxis.

Table 10. 5. Clinical manifestations of IgE-mediated reactions

Clinical features:
Dermatologic: Pruritus, flushing, urticaria/angioedema, diaphoresis
Eyes: Conjunctival injection, lacrimation, periorbital edema, pruritus
Respiratory tract: Nose/oropharynx (sneezing, rhinorrhea, nasal congestion, oral pruritus, metallic taste), upper airway (hoarseness, stridor, sense of choking, laryngeal edema), lower airway (dyspnea, tachypnea, wheezing, cough, cyanosis)
Cardiovascular: Conduction disturbances, tachycardia, bradycardia (if severe), arrhythmias, hypotension, cardiac arrest
Gastrointestinal: Nausea/vomiting, abdominal cramping, bloating, diarrhea
Neurologic: Sense of impending doom, syncope, dizziness, seizures

Urticaria and angioedema — Acute urticaria and angioedema are probably the most common cutaneous manifestations of food hypersensitivity reactions, generally appearing within minutes of ingestion of the food allergen. Food allergy may account for 20 percent of cases of acute urticaria.

By comparison, food allergies are an uncommon underlying cause of chronic urticaria and angioedema (defined as greater than six weeks of regular outbreaks).

Food can also cause acute contact urticaria. In this condition, urticaria develops only on skin that was in direct contact with the food. In addition to the common allergens, raw meats, seafood, raw vegetables and fruits, mustard, rice, and beer are among the foods that have been implicated in this form of reaction.

Oropharyngeal symptoms — Oropharyngeal symptoms can occur in isolation or as part of a systemic reaction to a food (table 10. 6). Symptoms may occur in isolation because the allergy is mild, not much allergen was ingested, or the allergen is labile, as is seen in oral allergy syndrome.

Oral allergy syndrome, or pollen-food allergy syndrome, is considered a form of contact allergy that is common in patients with allergic rhinitis to pollen. It is caused by the presence of heat-labile proteins (eg, profilins) within these foods that are cross reactive with allergenic pollen proteins. Symptoms are confined almost exclusively to the oropharynx, and include the immediate onset of pruritus, irritation, and mild swelling of the lips, tongue, palate, and throat upon ingestion of fresh, uncooked fruits and vegetables. The symptoms are not typically elicited by cooked fruits and vegetables. Symptoms usually subside within minutes after ingestion ceases. However, progression to systemic symptoms can occur and anaphylaxis has been reported. Symptoms may be more noticeable during the associated pollen season.

As examples, a birch-allergic patient may develop itching of the lips or mouth upon eating apple, pear, cherry, carrot, celery, and potato, while a ragweed-allergic patient may react to melons and banana, and a mugwort-allergic patient may react to celery or mustard. Tree nuts and peanuts can also cause isolated oral symptoms.

Respiratory tract symptoms — Asthma and environmental allergies (allergic rhinitis and conjunctivitis) are more common in children with food allergy. In addition, conjunctival, nasal, and lower respiratory tract symptoms are common components of systemic food allergic reactions (ie, anaphylaxis) (table 10. 7). However, isolated allergic rhinoconjunctivitis or asthma in response to foods is rare. An exception is occupational asthma (sometimes with accompanying rhinitis) in food industry workers. "Baker's asthma," caused by IgE-mediated allergy to inhaled wheat proteins, is an example. Patients with these conditions may not react to the food upon ingestion.

Gastrointestinal symptoms — IgE-mediated gastrointestinal (GI) symptoms, including nausea, abdominal pain, abdominal cramping, vomiting, and/or diarrhea, are more prominent features in anaphylaxis due to ingestion of a food allergen. The term gastrointestinal anaphylaxis is used when GI symptoms occur in isolation. However, GI symptoms are rarely the sole manifestations of a food-allergic reaction. More commonly GI symptoms occur in conjunction with involvement of other target organs (table 10. 6). The onset of upper gastrointestinal symptoms (nausea, vomiting, abdominal pain) is generally minutes to two hours after ingestion of the offending food, but lower gastrointestinal symptoms, such as diarrhea, can begin two to six hours after ingestion.

Anaphylaxis — Patients may develop a combination of symptoms and signs related to the cutaneous, respiratory, gastrointestinal, and/or cardiovascular systems that constitute anaphylaxis (table 10. 7). Anaphylactic reactions may culminate in hypotension, vascular collapse, cardiac dysrhythmias, or death. Anaphylaxis occasionally follows a biphasic course, with a recurrence of symptoms hours after the initial onset. Skin symptoms may be absent.

Food-dependent exercise-induced anaphylaxis — There are increasing reports of patients with anaphylaxis that occurs only if the patient exercises or exerts themselves within two to four hours of ingestion of food. This is referred to as food-dependent, exercise-induced anaphylaxis (FDEIAn). These reactions seem to be most prevalent in adolescents and young adults, although they can occur in middle aged patients as well.

Table 10. 7. Anaphylaxis signs and symptoms

Signs and symptoms of anaphylaxis:
Dermatologic : pruritus, flushing, urticaria, angioedema
Eyes : conjunctival injection, lacrimation, pruritus, periorbital edema
Nose : sneezing, rhinorrhea, nasal congestion
Upper airway : glossal/pharyngeal edema, metallic taste, hoarseness, stridor, sense of choking
Lower airway : dyspnea, tachypnea, wheezing, dry and repetitive cough, cyanosis
Gastrointestinal/abdominal : nausea, vomiting, crampy pain, diarrhea, uterine contractions (women)
Cardiovascular : hypotension, tachycardia (or sometimes bradycardia), palpitations, lightheadedness, syncope
Neurological : sense of impending doom

Most patients react to one or two specific foods. Common causative foods include wheat, celery, and seafood. The food can be ingested in the absence of exercise without development of symptoms. Some patients react after eating any food prior to exercise. These patients also have no reactions in the absence of exertion.

NON-IgE MEDIATED REACTIONS — Non-IgE mediated food allergies present as more subacute and/or chronic symptoms that are typically isolated to the gastrointestinal tract and/or skin. Affected patients commonly present with a characteristic constellation of clinical and demographic features that are consistent with well described disorders.

The exclusive non-IgE mediated food allergy disorders principally include:

- Food protein-induced enterocolitis syndrome (entire gastrointestinal tract)
- Food protein-induced enteropathy (small bowel)
- Food protein-induced proctitis and proctocolitis (rectum and colon)
- Celiac disease and dermatitis herpetiformis
- Food-induced pulmonary hemosiderosis (Heiner's syndrome)

The manifestations of these disorders are discussed briefly here and reviewed in detail elsewhere.

Gastrointestinal manifestations — The type of gastrointestinal signs and symptoms may vary in some disorders, depending upon whether the food is consumed regularly or infrequently. In addition, certain disorders are associated with systemic manifestations.

Passage of blood-tinged stools and mucus in an otherwise healthy breastfed infant without an anal fissure is suggestive of food protein-induced proctitis/proctocolitis. The most common trigger is cow's milk in the mother's diet. This disorder typically presents between two and eight weeks of age and resolves in a few days with complete elimination of the offending protein.

Chronic vomiting and diarrhea, particularly if accompanied by failure to thrive, suggests disorders such as food protein-induced enteropathy, celiac disease, food protein-induced enterocolitis syndrome (FPIES), or an eosinophilic gastrointestinal disorder.

Infants with FPIES are generally sicker in appearance than those with other non-IgE mediated allergic gastrointestinal disorders and they may have grossly bloody diarrhea (melena). The vomiting can be severe and can lead to dehydration. Patients may also have malabsorption. Laboratory abnormalities include hypoalbuminemia, anemia, and leukocytosis. Symptoms of FPIES resolve upon elimination of the causative food, although this may take several weeks (the symptoms should start to improve in at least one month).

If the causative food is later reintroduced, there is a characteristic delayed onset (approximately two to four hours) of profuse vomiting, followed by return of the other signs and symptoms. Children can require emergency treatment for hypotension, lethargy, or shock in this setting and laboratory studies may show acidosis, methemoglobinemia, and an increase in neutrophils. The acute manifestations of FPIES can be clinically identical to IgE-mediated gastrointestinal anaphylaxis, therefore testing is usually necessary to determine if food-specific IgE is present.

Older children and adults with FPIES typically present with a milder syndrome of nausea, protracted vomiting, and cramping several hours after ingestion.

FPIES is uncommon in exclusively breastfed infants. Cow's milk and soy are the most common triggers in infants and children, although many other food protein triggers have been reported. Shellfish is a common causative food in adults.

Infants with food protein-induced enteropathy can present with findings similar to patients with FPIES who are regularly ingesting a causative food (eg, chronic vomiting and diarrhea, failure to thrive).

Celiac disease, an enteropathy caused by gluten sensitivity, classically presents in infants and young children with chronic diarrhea, anorexia, abdominal distension and pain, failure to thrive or weight loss, and sometimes also vomiting. The gastrointestinal manifestations are similar in older children and adults,

but usually milder and include steatorrhea, weight loss, and other signs of nutrient or vitamin deficiency due to malabsorption. Flatulence and steatorrhea are suggestive of celiac disease rather than other forms of food-protein-induced enteropathy or FPIES.

Skin manifestations — The primary skin manifestation of exclusive non-IgE mediated food allergy is the vesicular eruption seen with dermatitis herpetiformis in patients with celiac disease. Dermatitis herpetiformis is characterized by an itchy papular vesicular eruption usually located symmetrically on the extensor surfaces of the elbows, knees, buttocks, sacrum, face, neck, trunk, and occasionally within the mouth. The predominant symptoms are itching and burning that are rapidly relieved with rupture of the blisters.

Pulmonary manifestations — Pulmonary involvement is a rare manifestation of non-IgE mediated food allergy.

Food-induced pulmonary hemosiderosis (Heiner's syndrome) is a rare syndrome in infants that consists of recurrent pneumonia with pulmonary infiltrates, hemosiderosis, iron deficiency anemia, and failure to thrive. Cow's milk is the most common causative food, with pork and egg also being reported. Elimination of the offending food results in resolution.

The pathogenesis of Heiner's syndrome is unclear. Serum precipitins to cow's milk and peripheral eosinophilia are often seen, and deposits of immunoglobulins and C3 may be found on lung biopsy. Lymphocytes from patients show abnormal proliferative responses to milk proteins.

Coexistence of celiac disease and idiopathic pulmonary hemosiderosis, also known as Lane-Hamilton syndrome, has been reported in a number of cases. Idiopathic pulmonary hemosiderosis is a rare disease found primarily in children that causes recurrent episodes of diffuse alveolar hemorrhage that may eventually produce pulmonary hemosiderosis and fibrosis. Diffuse alveolar hemorrhage is characterized by hemoptysis, dyspnea, alveolar opacities on chest radiographs, and anemia. Introduction of a gluten-free diet has been associated with remission of pulmonary symptoms in several patients.

MIXED IgE AND NON-IgE MEDIATED REACTIONS — Some food allergy disorders can have both IgE and non-IgE mediated components. Similar to the exclusively non-IgE mediated food allergies, the mixed disorders are typically isolated to the gastrointestinal tract and/or skin.

The mixed disorders primarily include:

- Atopic dermatitis
- Eosinophilic esophagitis
- Eosinophilic gastroenteritis

Food allergies may exacerbate atopic dermatitis, especially in young children with more severe eczema. Ingestion of the offending food acutely is thought to cause a flare of the patient's atopic dermatitis (increased erythema and pruritus of eczematous lesions). The flare occurs within minutes to a few hours if the reaction is IgE-mediated, but may take hours to days if the reaction is non-IgE mediated. The patient has persistent lesions if the food is eaten chronically.

The following features characterize the relationship between atopic dermatitis and food:

- The elimination of suspected food allergens frequently improves symptoms of atopic dermatitis within a few weeks
- Repeated exposure to suspect foods commonly exacerbates skin symptoms
- Eliminating foods to which an infant has demonstrable allergy can partially improve skin symptoms

The eosinophilic gastrointestinal disorders are characterized by symptoms of postprandial gastrointestinal dysfunction accompanied by eosinophilic infiltration of various segments of the intestinal tract on biopsy. The pathophysiology of the eosinophilic gastrointestinal disorders is poorly understood. Many patients have evidence of allergic sensitivities to foodand/or environmental allergens, but the causal role of these sensitivities is unclear.

Eosinophilic esophagitis (EoE) should be suspected in patients of any age presenting with esophageal symptoms. Infants and young children may present with feeding disorders, whereas older children and adults present with dysphagia, vomiting, and abdominal pain. A history of food impaction is common, particularly in adolescents and adults. Failure to respond to antacids and antireflux therapies is an important aspect of the history. Many patients with EoE have other atopic diseases. The most commonly implicated foods in children are cow's milk, egg, soy, corn, wheat, and beef, and most patients with evidence of food sensitivity tested positive for multiple foods. Elimination or elemental diets result in clinical and histologic improvement in most.

Eosinophilic gastroenteritis can present at any age with abdominal pain, nausea, diarrhea, malabsorption, and weight loss. In infants, it may present as outlet obstruction with postprandial projectile vomiting. In adolescents and adults, it can mimic irritable bowel syndrome. Symptoms vary depending on the layer and portion of the gastrointestinal tract that is involved. Approximately one-half of patients have allergic disease, such as defined food sensitivities, asthma, eczema, or rhinitis. However, in contrast to eosinophilic esophagitis, avoidance of implicated foods in those with an allergic food history has limited or no clinical benefit.

DIFFERENTIAL DIAGNOSIS — The differential diagnosis of food allergy includes a variety of disorders resulting from nonimmunologic reactions to food. As a group, these types of adverse food reactions are far more common than food allergy. Examples include lactose intolerance, gastroesophageal reflux, and disorders resulting from anatomic and neurologic abnormalities, enzymatic deficiencies, metabolic diseases, toxins, gastrointestinal infections, and a host of other processes (table 10. 5).

Allergic reactions to food additives are rare.

Migraine headache is another disorder that has not been linked to food allergy, although there are certain foods that can trigger migraines through nonallergic mechanisms due to their inherent chemical properties (eg, aromatic amine content) (table 10. 8).

Table 10. 8. Dietary triggers for migraine headache

Alcohol
Chocolate
Aged cheeses
Monosodium glutamate (MSG)
Aspartame (Nutrasweet)
Caffeine
Nuts
Nitrites, nitrates

ALLERGIC RHINITIS

Allergic rhinitis, or allergic rhinosinusitis, is characterized by paroxysms of sneezing, rhinorrhea, and nasal obstruction, often accompanied by itching of the eyes, nose, and palate. Postnasal drip, cough, irritability, and fatigue are other common symptoms.

Some investigators prefer the term rhinosinusitis to the separate terms rhinitis and sinusitis. This is because the nose and sinus mucosa are contiguous, rhinitis and sinusitis frequently occur together, rhinitis commonly leads to sinusitis, and nasal symptoms are common with sinusitis. However, within this topic

review, rhinitis and sinusitis are referred to separately, given that management issues may differ for each condition and detailed reviews of acute and chronic sinusitis are presented elsewhere.

The clinical manifestations, epidemiology, and diagnosis of allergic rhinitis are presented in this topic review. The pathogenesis and treatment of this condition are discussed separately.

EPIDEMIOLOGY — Allergic rhinitis is common, affecting 10 to 30 percent of children and adults in the United States and other industrialized countries. It may be less common in some parts of the world, although even developing countries report significant rates. The prevalence of asthma, rhinoconjunctivitis, and eczema were systematically evaluated in approximately 1.2 million children in 98 countries in the International Study of Asthma and Allergies in Childhood (ISAAC). The overall prevalence of rhinoconjunctivitis in children aged 6 to 7 years and 13 to 14 years was 8.5 and 14.6 percent, respectively.

The prevalence in the industrialized world is increasing, particularly in urban areas. Theories about the reasons for increasing prevalence are reviewed separately.

Economic burden — Allergic rhinitis is associated with significant morbidity and expense:

- It accounts for at least 2.5 percent of all clinician visits, 2 million lost school days per year, 6 million lost work days, and 28 million restricted work days per year.
- The average number of annual prescriptions for patients with allergic rhinitis is nearly double that for patients without allergic rhinitis (19 versus 10).
- Studies performed in the years around 2000 reported 2.4 billion American dollars spent on prescription and over-the-counter medications and $1.1 billion in clinician billings, causing a total indirect and direct cost of several billion dollars per year.

There is evidence that the economic burden is increasing, at least in the United States. Medical spending to treat allergic rhinitis almost doubled from 2000 to 2005 (6.1 to 11.2 billion dollars). In addition to costs directly attributable to allergic rhinitis, the disorder is highly associated with asthma and sinusitis, further expanding its economic impact.

RISK FACTORS — The following are proposed or identified risk factors for allergic rhinitis:

- Family history of atopy (ie, the genetic predisposition to develop allergic diseases)
- Male sex
- Birth during the pollen season
- Firstborn status
- Early use of antibiotics
- Maternal smoking exposure in the first year of life
- Exposure to indoor allergens, such as dust mite allergen
- Serum IgE >100 IU/mL before age six
- Presence of allergen specific IgE

A review of multiple studies reported that the presence of each of these factors was associated with a positive likelihood ratio for the diagnosis that ranged from three to five.

CLINICAL MANIFESTATIONS

Signs and symptoms — Allergic rhinitis presents with paroxysms of sneezing, rhinorrhea, nasal obstruction, and nasal itching. Postnasal drip, cough, irritability, and fatigue are other common symptoms. Some patients experience itching of the palate and inner ear. Those with concomitant allergic conjunctivitis report bilateral itching, tearing, and/orburning of the eyes.

Young children typically do not blow their noses, and instead, may repeatedly snort, sniff, cough, and clear their throats. Some scratch their itchy palates with their tongues, producing a clicking sound (palatal click).

Quality of life and cognitive function — Sleep disturbed breathing is one of the most important sequelae of untreated allergic rhinitis. Fatigue and generalized malaise are common, although patients

rarely report these symptoms directly. Allergic rhinitis is associated with a host of cognitive and psychiatric issues in children and adolescents, including attention deficit hyperactivity disorder, lower exam scores during peak pollen seasons, poor concentration, impaired athletic performance, and low self-esteem. In adults, allergic rhinitis is associated with anxiety, depression, reduced academic performance and work productivity (and lower than that of patients with asthma), impaired sexual performance, and lower quality of life scores.

Patterns of symptoms — Allergic rhinitis may be classified by temporal pattern (intermittent or persistent) and by severity (mild or moderate-severe) (table 10. 9):

- Intermittent — Symptoms are present less than four days per week or for less than four weeks
- Persistent — Symptoms are present more than four days per week and for more than four weeks
- Mild — None of the items listed below for "moderate-severe" are present
- Moderate-severe — One or more of the following items is present:
- Sleep disturbance
- Impairment of school or work performance
- Impairment of daily activities, leisure and/or sport activities
- Troublesome symptoms

Table 10. 9. Classification of allergic rhinitis

"Intermittent" means that the symptoms are present: • Less than four days a week • Or for less than four weeks
"Persistent" means that the symptoms are present: • More than four days a week • And for more than four weeks
"Mild" means that none of the following items are present: • Sleep disturbance • Impairment of daily activities, leisure and/or sport • Impairment of school or work • Troublesome symptoms
"Moderate-severe" means that one or more of the following items are present: • Sleep disturbance • Impairment of daily activities, leisure and/or sport • Impairment of school or work • Troublesome symptoms

This classification system was proposed by an international workshop of 34 specialists in respiratory allergy, in collaboration with the World Health Organization (WHO). The WHO had targeted allergic rhinitis because of its impact on asthma and mirrors the consensus asthma guidelines.

Other commonly used terms are “seasonal”, which is allergic rhinitis that occurs at a particular time of the year (figure 10. 2), and “perennial,” which describes symptoms to allergens that are present year round. This system of classification is preferred by the US Food and Drug Administration (FDA).

Patients whose symptoms occur episodically are often more aware of the disability caused by allergic rhinitis, whereas patients with chronic symptoms often adapt to significant impairment over time,

and may not seek medical care until symptoms become severe. Children, in particular, will endure significant disability.

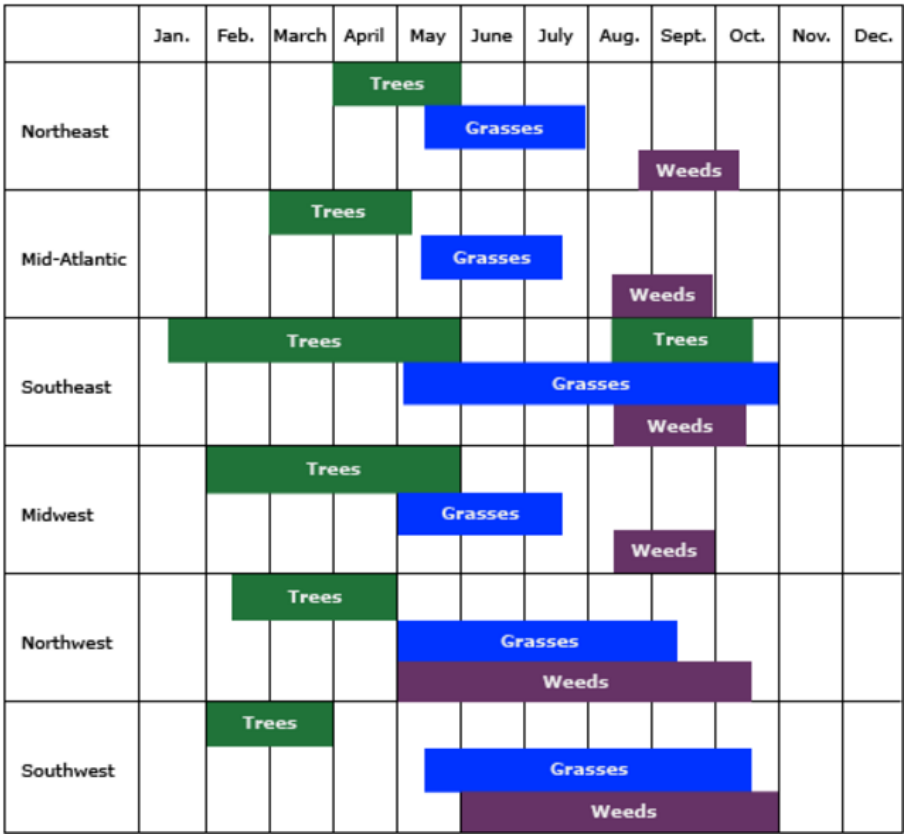

Figure 10. 2. Peak pollen periods in the United States.

Persistent/perennial symptoms are more common than purely intermittent or seasonal symptoms, although many patients have perennial symptoms with seasonal exacerbations.

Seasonal allergy rhinitis is usually caused by pollen from trees, grasses, and weeds. Depending upon the geographic area, pollination periods for certain types of plants are well known (figure 1). Colloquial names sometimes correctly identify the triggering pollen (eg, cedar fever), although in other cases, a plant that is not causing symptoms but is very visible at the same time is implicated by the name (eg, rose fever, which refers to allergic rhinitis caused by grasses that pollinate at the same time that roses bloom, or hayfever, which occurs in the fall when hay is being gathered, but is caused by weed pollens or from mold growing in the hay). Symptoms of seasonal allergic rhinitis are predictable and reproducible from year to year.

Perennial allergic rhinitis usually reflects allergy to indoor allergens like dust mites, cockroaches, mold spores, or animal dander, although aeroallergens may cause perennial rhinitis in tropical or subtropical climates. Perennial rhinitis due to outdoor allergens is common in subtropical regions with long pollinating seasons and ever present mold and dust mite allergens, and with occupational allergen exposure.

It is important to understand that allergic rhinitis, whether seasonal or perennial, may be difficult to distinguish clinically from the nonallergic forms of rhinitis, since not all seasonal and perennial symptoms are unique to allergic forms of rhinitis. As an example, chronic nonallergic rhinitis can be triggered by changes in weather and temperature, and may appear to have a seasonal pattern in some patients. Thus, for an accurate diagnosis of allergic rhinitis, diagnostic testing may be required.

Increasing sensitivity over time — When a patient is continually exposed to an allergen, persistent nasal mucosal inflammation develops. In such patients, symptoms of rhinitis occur on exposure to lower doses of allergen (priming) and to nonspecific irritants (hyperreactivity). Clinically, this results in

continued and frequently more severe rhinitis symptoms with exposure to low allergen concentrations. This phenomenon of increasing sensitivity over time probably results from a lowering of the threshold for a clinical response. Sequential allergen challenges in patients with allergic rhinitis result in more symptoms and higher levels of histamine and inflammatory cells in nasal washes. Presumably, the induced inflammation recruits additional inflammatory cells and their products into the process, producing increasing symptoms.

Concomitant with allergen priming, the nose becomes progressively more sensitive to cholinomimetic stimuli, such asmethacholine and histamine, as well as irritant stimuli like cold air. Over time, many patients with allergic rhinitis report heightened sensitivity to irritants (eg, tobacco smoke, particulate pollution), volatile substances, and strong scents and perfumes.

Physical findings — The following physical findings may be present in patients with active allergic rhinitis:

- Infraorbital edema and darkening due to subcutaneous venodilation, findings that are sometimes referred to as "allergic shiners"
- Accentuated lines or folds below the lower lids (Dennie-Morgan lines), which suggests concomitant allergic conjunctivitis
- A transverse nasal crease caused by repeated rubbing and pushing the tip of the nose up with the hand (the "allergic salute")
- "Allergic facies," which are typically seen in children with early-onset allergic rhinitis, consist of a highly arched palate, open mouth due to mouth breathing, and dental malocclusion The internal structures of the nose, oropharynx, and ears should be examined:
- The nasal mucosa of patients with active allergic rhinitis frequently has a pale bluish hue or pallor along with turbinate edema
- Clear rhinorrhea may be visible anteriorly, or if the nasal passages are obstructed, rhinorrhea may be visible dripping down the posterior pharynx
- Hyperplastic lymphoid tissue lining the posterior pharynx, which resembles cobblestones (a finding called “cobblestoning”)
- Serous fluid may accumulate behind tympanic membranes in patients with significant nasal mucosal swelling and eustachian tube dysfunction

Routine laboratory findings — Routine laboratories are usually normal. Neither peripheral blood eosinophil counts nor total serum IgE levels (elevated in only 30 to 40 percent of patients) are sensitive enough to help diagnose allergic rhinitis.

Natural history — Allergic rhinitis typically requires a few years of allergen exposure to develop. Accordingly, it is uncommon in children under two years of age. If a very young child appears to have persistent nasal symptoms, other disorders should be considered.

Sensitization to aeroallergens generally precedes the appearance of rhinitis symptoms. Sensitization describes the presence of allergen-specific IgE, as measured by skin testing or in vitro tests. However, sensitization is not synonymous with allergy, and a person can be sensitized to an allergen without developing symptoms when exposed to that allergen. Only a subset of sensitized individuals demonstrate clinical allergy. The distinction between sensitization and clinical allergy is discussed in more detail elsewhere.

In children, sensitization and then clinical allergy develop first to allergens that are continually present in the environment (eg, dust mites or animal danders) and then to pollens and other seasonal allergens. In a study of approximately 600 children, at least two seasons of pollen exposure were required before most children developed allergic symptoms.

After the age of two, the prevalence of allergic rhinitis steadily increases, demonstrating a bimodal peak in the early school and early adult years. In a prospective study of 2024 children, the prevalence of

allergic rhinitis increased from 5 percent at the age of four years, to 14 percent at age eight. The prevalence then gradually increases, peaking in early adulthood. The condition is usually persistent throughout adulthood, with some improvement in older age.

Allergic rhinitis uncommonly presents for the first time in older adults, unless there is a significant change in exposures (eg, a new pet or a move to a different climate). Thus, in an older adult with new nasal symptoms, other causes of rhinitis should be considered. A change in response to treatment in older adults may indicate the development of vasomotor or atrophic rhinitis.

ASSOCIATED CONDITIONS — Allergic rhinitis occurs in association with a number of other disorders, including allergic conjunctivitis, acute or chronic sinusitis, asthma, and atopic dermatitis (eczema).

- **Allergic conjunctivitis:** Up to 60 percent of patients with allergic rhinitis have concomitant allergic conjunctivitis. Allergic conjunctivitis presents with itching, tearing, conjunctival edema, hyperemia, watery discharge, burning, and photophobia. Eyelid edema is also common. Symptoms are usually bilateral.
- **Sinusitis:** Nasal inflammation associated with allergic rhinitis can also cause obstruction of the sinus ostiomeatal complex, thereby predisposing to bacterial infection of the sinuses. This process accounts for as much as 30 to 80 percent of cases of acute and chronic bacterial sinusitis, respectively. However, purulent sinusitis without concurrent rhinitis is rare, given that these tissues are anatomically contiguous. Symptoms of bacterial sinusitis include nasal congestion, purulent rhinorrhea or postnasal drip, facial or dental pain, and cough. Purulent rhinorrhea, purulent postnasal drip, or pain in a maxillary tooth and persistent cough in children are the most useful predictors of bacterial sinusitis. However, no single symptom has a high degree of sensitivity or specificity in discriminating bacterial sinusitis from allergic or viral rhinitis.
- **Asthma:** Up to 50 percent of patients with asthma have allergic rhinitis. In children, cough and wheeze are the most common symptoms, and are often more prominent with exertion (table 10. 10). Adults may report any combination of cough, wheeze, or chest tightness. The relationships between asthma and allergic rhinitis are discussed in detail separately.
- **Atopic dermatitis (eczema)** : In children, atopic dermatitis presents with intensely pruritic erythematous patches with papules and some crusting, usually affecting the face, scalp, extremities, or trunk, with sparing of the diaper areas. In older individuals with more chronic disease, atopic dermatitis presents with thickened skin, increased skin markings (lichenification), and excoriated and fibrotic papules. The flexural areas (neck, antecubital fossae, and popliteal fossae) are most commonly involved in adults. This disorder is discussed separately.
- **Oral allergy syndrome** : Oral allergy syndrome is a form of food allergy that develops in individuals who are sensitized to pollens. Patients report itching and/or mild swelling of the mouth and throat immediately following ingestion of certain uncooked fruits (such as apples, peaches, plums, cherries, and some nuts) or raw vegetables. The symptoms result from contact urticaria in the oropharynx caused by pollen-related proteins in these foods.
- **Other conditions** : Allergic rhinitis is strongly associated and probably causally related to eustachian tube dysfunction, causing concomitant serous and acute otitis media. Nasal obstruction due to severe allergic rhinitis can also cause sleep disordered breathing and anosmia. There may be an increased prevalence of migraine headache in patients with allergic rhinitis.

DIAGNOSIS. The diagnosis of allergic rhinitis can be made on clinical grounds based upon the presence of characteristic symptoms (ie, paroxysms of sneezing, rhinorrhea, nasal obstruction, nasal itching, postnasal drip, cough, irritability, and fatigue), a suggestive clinical history (including the presence of risk factors), and supportive findings on physical examination. Allergy skin testing confirms that the patient is sensitized to aeroallergens, although it is not necessary for the initial diagnosis.

Table 10. 10. Sample questions* for the diagnosis and initial assessment of asthma

A "yes" answer to any question suggests that an asthma diagnosis is likely.
In the past 12 months...
Have you had a sudden severe episode or recurrent episodes of coughing, wheezing (high-pitched whistling sounds when breathing out), chest tightness, or shortness of breath?
Have you had colds that "go to the chest" or take more than 10 days to get over?
Have you had coughing, wheezing, or shortness of breath during a particular season or time of the year?
Have you had coughing, wheezing, or shortness of breath in certain places or when exposed to certain things (eg, animals, tobacco smoke, perfumes)?
Have you used any medications that help you breathe better? How often?
Are your symptoms relieved when the medications are used?
In the past 4 weeks, have you had coughing, wheezing, or shortness of breath...
At night that has awakened you?
Upon awakening?
After running, moderate exercise, or other physical activity?

** These questions are examples and do not represent a standardized assessment or diagnostic instrument. The validity and reliability of these que*

Imaging is not usually performed in the diagnosis of allergic rhinitis, unless a concomitant condition, such as chronic rhinosinusitis, is suspected or there is a history of facial trauma or features to suggest anatomic abnormalities (unilateral congestion or obstruction).

If the patient's symptoms prove difficult to manage or the trigger(s) for the symptoms are not apparent, then further evaluation is indicated to demonstrate that the patient is sensitized to aeroallergens and that symptoms occur when expected for the allergens in question. Sensitization can be demonstrated with either allergy skin testing or in vitro tests for allergen-specific IgE.

A positive response to a therapeutic trial of either topical nasal steroids or topical antihistamines does not conclusively establish a diagnosis of allergic rhinitis, because these therapies are also effective in the treatment of nonallergic rhinitis.

History — Some forms of allergic rhinitis can be readily diagnosed by history alone, while others may require additional evaluation for accurate diagnosis:

- Seasonal allergic rhinitis is often diagnosed by the history alone, because it is reproducible from year to year. Seasonal allergic rhinitis caused by tree and grass pollen typically occurs in the spring, and symptoms caused by ragweed pollen exposure in the fall, although there are regional variations (figure 10. 2).
- Some cases of episodic allergic rhinitis can be diagnosed by history alone if there is an obvious connection between exposure and the onset of symptoms. As an example, a history of episodic exposure to animals or high levels of house dust resulting in acute allergic symptoms may be readily diagnosed as episodic allergic rhinitis.

- By comparison, the culprit allergens in perennial/persistent allergic rhinitis may not be readily apparent from the clinical history. Perennial allergic rhinitis usually reflects allergy to indoor allergens like dust mites, cockroaches, or animal dander, although pollens may cause perennial rhinitis in tropical or subtropical climates.

Physical examination — The nose, oropharynx, tympanic membranes, and eyes should be examined, as each of these structures may show findings of allergic rhinitis or associated disorders.

The internal structures of the nose and the nasal mucosa can be visualized using a standard office otoscope with a disposable tip. Stabilizing the tip against the patient's upper nares prevents painful prodding of the mucosa, which is normally exquisitely sensitive to touch.

In patients older than five years of age, flexible fiberoptic rhinoscopy is helpful but not essential for diagnosis. If pursued, it should be performed by clinicians specifically trained in the technique. This technique is described elsewhere.

Allergen-specific testing — It is not necessary to perform testing for allergen-specific IgE, either with blood tests or skin testing, before making the presumptive diagnosis of allergic rhinitis and initiating treatment. Primary care clinicians treat the majority of patients with allergic rhinitis, and often initiate therapy empirically, identifying possible triggers only through the clinical history. This approach is adequate for many patients.

Despite the above, the use of diagnostic testing to identify culprit allergens has been associated with improved patient outcomes. Identifying the allergens that are important to an individual facilitates avoidance of the allergen and identifies candidates for allergen immunotherapy, which can eventually reduce reliance on chronic medications.

Properly performed skin testing is the most convenient, sensitive, and least expensive screening method to detect allergic sensitization. Skin testing is usually performed by allergy experts because, although generally considered a safe technique, rare systemic allergic reactions are possible in response to the testing itself.

Skin testing — Immediate hypersensitivity skin testing (prick skin tests) is a quick, cost effective, and safe way to identify the presence of allergen-specific IgE. These tests are usually performed by allergy specialists. In sensitive patients, testing with selected diagnostic solutions of tree, grass, or weed pollen, mold, house dust mite, and/oranimal allergens results in a wheal and flare reaction at the skin test site within 20 minutes. Positive prick skin tests correlate more closely with symptoms than intradermal tests.

Skin testing is particularly useful among patients with:

- An unclear diagnosis based upon the history and physical examination
- Poorly controlled symptoms, such as persistent nasal symptoms and/or an inadequate clinical response to nasal glucocorticoids
- Coexisting persistent asthma and/or recurrent sinusitis/otitis
- A high pre-test probability of allergic rhinitis and negative in vitro test results to suspected culprit allergens (because the sensitivity of skin testing is usually superior to that of in vitro testing)
- A patient's expressed desire to try to avoid the allergen rather than take medications to control symptoms

Skin testing a very symptomatic pollen-allergic patient during peak pollen season should be avoided, as it can aggravate symptoms further and may be associated with higher rates of systemic reactions during testing. In this setting, the patient's symptoms should be treated empirically and testing should be deferred until the patient is less symptomatic.

Serum tests for allergy — Immunoassays to detect allergen-specific IgE antibodies in the serum have limited utility in the diagnosis of allergic rhinitis. These tests are sometimes referred to as

radioallergosorbent tests, or RASTs, because earlier methods utilized radioactive reagents. The methods currently in use are more correctly referred to as immunoassays for allergen-specific IgE.

IgE immunoassays provide similar information as that obtained with allergen skin tests, although they are more expensive and less sensitive for the diagnosis of allergy to inhalant allergens, compared with skin tests. The precise sensitivity of IgE immunoassays, compared to skin prick testing, has been reported to range from less than 50 percent to more than 90 percent, with the average being 70 to 75 percent in most studies. Similar sensitivity ranges apply when immunoassay results were compared with symptoms induced during natural or controlled target organ challenge procedures.

The utility of screening panels of IgE immunoassays has been evaluated in the diagnosis of allergic rhinitis. Such testing can improve diagnostic accuracy and/or management, when skin testing is not available. These panels typically test for IgE antibodies to common seasonal and perennial allergens, and are intended for use by generalists. However, this approach can be costly if excessive numbers of immunoassays are measured, or if the allergens selected are not relevant to the geographic area in question. A logical approach would involve consulting an allergy expert in the area initially to identify a small number of important allergens for the area (eg, a few prominent pollens, animal danders, molds for dry environments and dust mites for humid environments).

Suggestive history with negative testing — Occasionally, a patient presents with a history and physical findings suggestive of allergic rhinitis, but skin testing and in vitro testing are negative. Most commonly, such patients have chronic nonallergic rhinitis, which has subtly different historical features and physical findings, as reviewed below.

Another possibility is that the patient is producing allergen-specific IgE locally in the nasal tissues, but it is not reflected in the systemic circulation or skin. This condition is sometimes referred to as “local allergic rhinitis” and it is an area of increasing research interest. Local allergic rhinitis is discussed separately.

Fortunately, patients with either of these disorders may be managed similarly to those with allergic rhinitis.

Uncommonly used tests — Nasal cytology and direct inhalational challenge with allergen are techniques that are largely limited to research settings.

Nasal cytology — Nasal cytology is performed by some investigators to help differentiate rhinitis due to allergy from that due to infection, although it is relatively nonspecific and insensitive. Nasal secretions may be obtained with a cotton swab, or by asking the patient to blow the nose onto waxed paper or cellophane. Wright stain of nasal secretions usually, but not always, reveals a predominance of eosinophils in cases of allergic rhinitis. By comparison, the presence of neutrophils suggests an infectious process.

Nasal eosinophilia may also be seen in other conditions including:

- Asthma without symptoms of nasal allergy
- Nasal polyposis, with or without asthma and aspirin sensitivity
- Nonallergic rhinitis with eosinophilia syndrome (NARES), a syndrome of marked nasal eosinophilia and a propensity for nasal polyps, but with negative allergic histories, negative skin tests, and no aspirin sensitivity

Some also utilize nasal cytology to assess the response to antiinflammatory medications, thereby providing further support for a particular diagnosis. Eosinophilia, for example, should decrease with therapy in patients with allergic rhinitis.

Allergen challenge — Although nasal allergen challenge can definitively establish the diagnosis, it is clinically impractical and rarely performed outside of research settings. Nasal challenge procedures are discussed elsewhere.

Unproven diagnostic tests — There are several unproven or inappropriate diagnostic testing assays that are being offered by various types of providers with increasing frequency. These include cytotoxic testing, provocation-neutralization testing, and specific or nonspecific IgG determinations. The results of these methods are **not** useful for diagnosis or management.

DIFFERENTIAL DIAGNOSIS — Various other disorders can mimic allergic rhinitis or coexist with it in older children and adults.

Children under two years of age — Because allergic sensitization takes a few years to develop, other disorders should be considered in very young children who have persistent rhinitis symptoms.

These include adenoidal hypertrophy, acute or chronic sinusitis, congenital abnormalities (choanal atresia), foreign bodies, and nasal polyps.

Older children and adults — Rhinitis can result from either inflammatory or noninflammatory causes.

- **Acute infectious rhinitis** : Symptoms of the common cold vary from patient to patient, with rhinitis and nasal congestion being the most common. Nasal obstruction, rhinorrhea, and sneezing are usually present early in the course of the cold, although a sore or "scratchy" throat is frequently the most bothersome symptom on the first day of illness. The sore throat is usually short lived, and nasal symptoms predominate by the second and third day. Cough typically becomes troublesome on the fourth or fifth day of illness, by which time the nasal symptoms are less severe. Acute bacterial sinusitis develops in 0.5 to 2.5 percent of adult patients after viral upper respiratory tract infection. Viral sinusitis occurs much more frequently. The clinical presentation of the patient is of limited utility in distinguishing cases of pure viral rhinosinusitis from those with secondary bacterial infection. There appear to be **no**signs and symptoms of acute respiratory illness that are both sensitive and specific in making this distinction. Symptoms and/or complaints of a common cold that last for more than two weeks may be confused with those due to allergic rhinitis. As previously mentioned, however, many patients commonly have both sinusitis and allergic rhinitis, a setting in which the diagnosis and treatment of both conditions must be entertained. The remaining disorders discussed below result from chronic processes.

- **Chronic nonallergic rhinitis** : Approximately 50 percent of patients with chronic rhinitis have a component of nonallergic rhinitis. Chronic nonallergic rhinitis is characterized by perennial symptoms and mild or absent nasal itching and sneezing. Patients with this disorder complain of chronic nasal congestion and/or rhinorrhea that is intensified by rapid changes in temperature and relative humidity, odors, or alcohol. They have little nasal itching or sneezing; however, headaches, anosmia, and sinusitis are common. A family history of allergy or allergic symptom triggers is uncommon. Negative skin tests to inhalant allergens are essentially diagnostic for a nonallergic rhinitis syndrome. Syndromes of nonallergic rhinitis include vasomotor rhinitis, gustatory rhinitis, and nonallergic rhinitis with nasal eosinophilia syndrome. These are reviewed in more detail separately

- **Chronic rhinosinusitis** : Chronic rhinosinusitis (CRS) is defined as an inflammatory condition involving the paranasal sinuses and linings of the nasal passages that lasts 12 weeks or longer, despite attempts at medical management. CRS can coexist with allergic rhinitis. The diagnosis of CRS requires any TWO of the following symptoms, present for at least 12 weeks:
  - Anterior and/or posterior mucopurulent drainage
  - Nasal obstruction
  - Facial pain, pressure, and/or fullness
  - Decreased sense of smell

Concomitant CRS should be considered when a patient with allergic rhinitis also has the above-mentioned symptoms and fails to improve with treatment for allergic rhinitis. The diagnosis of CRS is discussed in greater detail elsewhere.

Rhinitis medicamentosa : Rhinitis medicamentosa is a complication of vasoconstrictor nasal sprays (which may develop with as little as five days of use) or intranasal cocaine abuse. Chronic nasal obstruction and nasal inflammation develop and are manifested as beefy red nasal membranes on physical examination. Diagnosis depends almost entirely on the appropriate history, characteristic physical examination findings, and positive response to treatment with topical nasal steroids, which is usually required to withdraw successfully from the culprit medication.

- **Rhinitis due to systemic medications** : A variety of systemic medications can induce nasal symptoms. This is in contrast to rhinitis medicamentosa, which is a specific syndrome that results from the intranasal application of certain drugs. Classes of medications that can cause nasal symptoms include birth control pills, antihypertensive drugs (alpha-adrenergic blockers, beta-adrenergic blockers, ACE inhibitors), erectile dysfunction drugs, and nonsteroidal antiinflammatory drugs. Psychiatric medications that have been implicated include chlorpromazine , thioridazine ,perphenazine , chlordiazepoxide , amitriptyline , and alprazolam . Lastly, the immunosuppressants cyclosporine and mycophenolic acid can cause nasal symptoms. Rhinitis symptoms caused by these medications generally subside within a few weeks of discontinuation.

- **Atrophic rhinitis** : Atrophic rhinitis is a syndrome of progressive atrophy of the nasal mucosa usually seen in elderly patients; such individuals report chronic nasal congestion and perceive a persistent bad odor. This condition is associated with mucosal colonization with Klebsiella ozaenae. A variant occurs in patients who have had multiple sinus surgeries resulting in loss of normal mucociliary function..

- **Rhinitis associated with hormonal changes** : Rhinitis of pregnancy and rhinitis of hypothyroidism reflect nasal obstruction that occurs on a hormonal basis. In these settings, the diagnosis is clinical and is supported by negative skin tests or improved symptoms upon resolution or treatment of the causative condition.

- **Unilateral rhinitis or nasal polyps** : Unilateral rhinitis or nasal polyps are uncommon in uncomplicated allergic rhinitis. Unilateral rhinitis suggests the possibility of nasal obstruction by a foreign body, tumor, or polyp, and the presence of nasal polyps suggests NARES, chronic bacterial sinusitis, allergic fungal sinusitis, aspirinhypersensitivity, cystic fibrosis, or primary ciliary dyskinesia (immotile cilia syndrome). Fiberoptic rhinoscopy may be helpful in this setting. Increasing evidence also suggests that the histology of those with rhinitis with or without nasal polyps are different, with eosinophils or neutrophils predominating in those with or without polyps, respectively. However, allergic rhinosinusitis and NARES are both conditions characterized by nasal eosinophilia, but most patients with these conditions lack nasal polyps.

- **Rhinitis with immunologic disorders** : A number of systemic autoimmune disorders present with nasal symptoms or can affect nasal mucosa. These include granulomatosis with polyangiitis (Wegener's) and relapsing polychondritis:

- The most common presenting symptoms of granulomatosis with polyangiitis include persistent rhinorrhea,purulent/bloody nasal discharge, oral and/or nasal ulcers, polyarthralgias, myalgias, or sinus pain.

- With relapsing polychondritis, symptoms of stuffiness, crusting, rhinorrhea, and on occasion, epistaxis, may accompany nasal cartilage inflammation, which can also compromise olfaction. Cartilage destruction associated with sustained or recurrent episodes of inflammation can result in a characteristic saddle nose deformity. Such disorders may therefore present with

nasal symptoms, without evidence of systemic disease. These disorders are diagnosed based upon the combination of characteristic histologic and clinical findings.

CONTACT DERMATITIS

Contact dermatitis is one of the most common inflammatory skin diseases in the industrialized world. Repeat exposure to chemicals in the environment, both at home and in the workplace, has resulted in a significant increase in cutaneous pathologic responses. It is a major health concern for patients and has a major impact on the economy. A recent study in the UK identified that occupational skin disease accounts for approximately 20% of all work-related health complaints, resulting in an estimated 4 million lost work-days and bringing an associated cost of almost £200 million per year. In the USA, the rates are lower but this is believed to be due to variations between countries in reporting occupational diseases. For example, the Bureau of Labor Statistics only include a representative random sample of employees in private industry, suggesting that occupational contact dermatitis is underreported. A direct extrapolation of the UK data to the USA, based on population alone, suggests the impact of occupational contact dermatitis at almost US$2 billion per year.

Classification and etiology

Contact dermatitis represents the vast majority (79–90% annually) of skin-related occupational complaints. It can be divided into four categories based on etiology. Irritant contact dermatitis (ICD) is the most prevalent form, commonly thought to cause 80% of cases of contact dermatitis. An irritant will cause direct injury to the skin in any person if applied in a sufficient concentration for a sufficient amount of time. No previous sensitization is required, and can be induced by exposure to toxic chemicals, such as acids (coagulate epidermal proteins), oxidants (change the integrity of the epidermis), and alkali, surfactants, and solvents (remove protective surface lipids) that result in toxicity to epidermal cells leading to an inflammatory response.

A second category is photocontact dermatitis in which a chemical requires light-induced excitation in the ultraviolet spectrum to cause a cutaneous response. These compounds may be photoallergic or phototoxic and can be induced from either topical or systemic exposure. Photoallergic reactions require prior sensitization to the compound and common topical offending agents include musk ambrette, para-aminobenzoic acid (PABA), plants of the Compositae family, chlorhexidine, and thiourea. Systemic medications capable of causing photoallergy are non-steroidal antiinflammatory agents (NSAIDs), phenothiazines, thiazide diuretics, dapsone, and sulfonylureas. Phototoxic reactions do not require prior sensitization and can be induced topically by psoralens, furocoumarins, tar, lime, celery, and parsnip; and systemically by antibiotics, especially the tetracyclines, amiodarone, diuretics, quinine, and NSAIDs. Dermatitis may develop after exposure to UVA or UVB light, with each compound requiring activation by a specific wavelength spectrum.

A third major category is contact urticaria. It is characterized as a wheal-and-flare response and can be either immunologic (IgE-dependent) or non-immunologic (IgE-independent). The lists of agents causing immunologic contact urticaria is extensive and includes dairy products, seafood, various fruits, grains, topical antibiotics, metals, fragrances, preservatives, and plants (algae, chrysanthemum, lilies, tulips). Many agents cause non-immunologic contact urticaria (for example, fragrances, arthropods, jellyfish, and coral).

The final category is allergic contact dermatitis (ACD), also known as contact hypersensitivity (CHS). It is generally accepted that the pathogenesis of ACD involves a complex immune-mediated process made up of two distinct phases in response to exposure to environmental chemicals, the induction (also known as the afferent or primary) and the elicitation (also known as the efferent or secondary) phases. The details of this mechanism will be discussed in detail later in the chapter. A large number of compounds are known to cause allergic contact dermatitis. The sap of the poison ivy and poison oak

plants contains urushiol, with a very high percentage of the population being allergic as a result of sensitizing skin exposures to the resin derived from this plant. Notably, nickel is the most common allergen routinely found during patch testing, and is found in jewelry, coins, snaps, and buttons. The dimethylgloxime test can be used to rapidly determine if a given metal contains nickel. Neomycin and bacitracin are antibiotics that are commonly used in creams, ointments, and eye drops. Balsam of Peru is a naturally occurring compound extracted from the *Myroxylon pereirae* tree, and it is used in fragrances, pharmaceuticals, and flavorings. Thimerosal is a preservative used in cosmetics, contact lens solution, vaccines, and topical medicaments. Patch testing is a commonly used tool to determine which allergens may be causing contact dermatitis.

Clinical features

There may be overlap in the clinical presentation of the different types of contact dermatitis. For example, irritant contact dermatitis and allergic contact dermatitis of the hands may have a similar presentation. Clues to diagnosis include the distribution, morphology, and timing of the eruption. It is also important to ask about the associated symptoms, aggravating factors, and response to treatment. A detailed history is usually necessary to determine the type of contact dermatitis and the possible etiologic agents.

Allergic contact dermatitis can occur anywhere on the body, depending on the allergen involved. For example, the frequent sites of nickel allergy are on the abdomen from snaps on pants or buckles, and the wrists and earlobes from jewelry. Acute contact dermatitis manifests as erythematous papules, edema, and vesiculation (Fig. 10.3). There may be extreme pruritus and the lesions may spread beyond the areas of initial contact. Chronic dermatitis will show lichenification and possibly hyperpigmentation of the skin. Hand dermatitis is common, comprising about 20–35% of cases of dermatitis. There can be additional fissuring of the hands, and it is usually bilateral. In many cases, hand dermatitis is a result of occupational exposure and can be caused by an irritant.

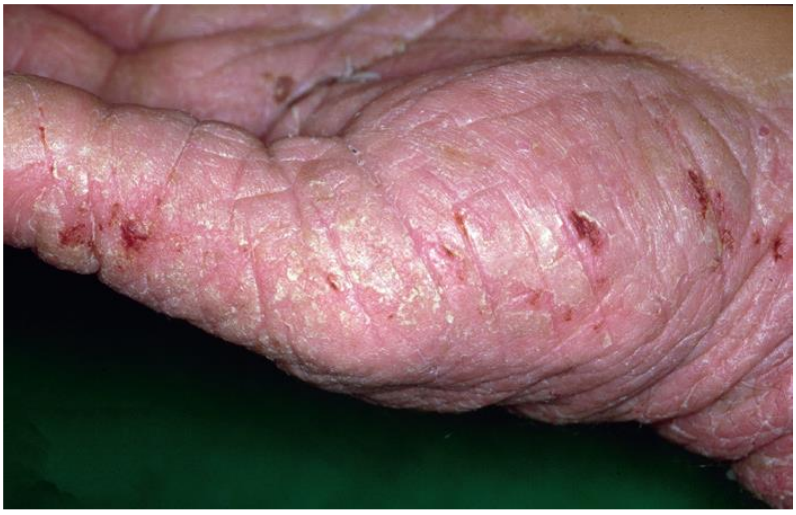

Fig. 10.3 Acute contact dermatitis. Note the erythematous, edematous papules and vesicles. This reaction was to bacitracin applied topically after surgery.

Several characteristics can differentiate ICD from ACD. Irritant contact dermatitis tends to have more of a stinging characteristic or mild pruritus versus severe pruritus of ACD. Reactions can be almost immediate with irritants, whereas with ACD these reactions take usually 12–48 h to develop. A negative patch test with relevant allergens test makes ICD more likely. Those that perform ‘wet work’, spending a significant amount of time with wet hands, and the use of irritating compounds such as soap, are more likely to suffer from ICD. Irritant contact dermatitis and allergic contact dermatitis have significant clinical overlap, and the physician must use the pertinent history and physical examination, as well as patch test result, to characterize the eruption.

Contact urticaria manifests clinically as transient erythematous, edematous plaques, known as the wheal-and-flare response. The lesions occur at the site of exposure to an allergen within minutes to hours of contact with associated pruritus. They resolve within hours and leave normal-appearing skin. Photoallergic and phototoxic reactions occur in the sun-exposed parts of the body. Common sites are the face, V-portion of the neck, arms, and hands. The submental neck may be spared, as well as the medial arms or areas covered by jewelry. Phototoxic reactions take the form of exaggerated sunburn, with erythema and vesiculation. Typically, photoallergic reactions have an eczematous appearance, with erythematous papules, edema, and vesicles. Chronically, photoallergic dermatitis will demonstrate lichenification and hyperpigmentation. The different types of contact dermatitis can be differentiated based on history taking, physical examination, and pertinent patch test results.

Patch testing

If ACD is suspected, patch testing offers an objective test to determine the specific allergen that may be causing the dermatitis. Patients must be willing to return for three total office visits and need to abstain from bathing or heavy exercises while the patches are in place. At the time of patch testing, the patient's dermatitis must be under excellent clinical control. That is, the dermatitis cannot be severe or acute, as this may result in the false-positive, ‘angry back’ reaction, in which there are multiple strong positive patch test reactions that are not reproducible on repeat patch testing. Preferably, patients should not be taking high-dose systemic corticosteroids (low dose, up to 10–20 mg/day, may be acceptable). It is also advisable to avoid the application of potent topical corticosteroids to the patch test sites on the back because this may also cause a false-negative result. Once the patient is deemed to be a patch test candidate and is counseled on the process, possible allergens are placed in Finn chambers and affixed to the back of the patient with tape. The tape is kept in place for 48 h and the patient returns to the office for the initial reading, and also in 96 h for the final reading. Each area corresponding to a different allergen is read, a ± reaction has only macular erythema, a 1+ reaction consists of erythema and edema, 2+ reaction contains papules, and a 3+ reaction has vesicles or bullae. No evidence of skin changes is considered a negative reaction (Fig. 10.4). Several standard trays are available, including the North American standard series, as well as specialized sets, such as metal, dental, or fragrance.

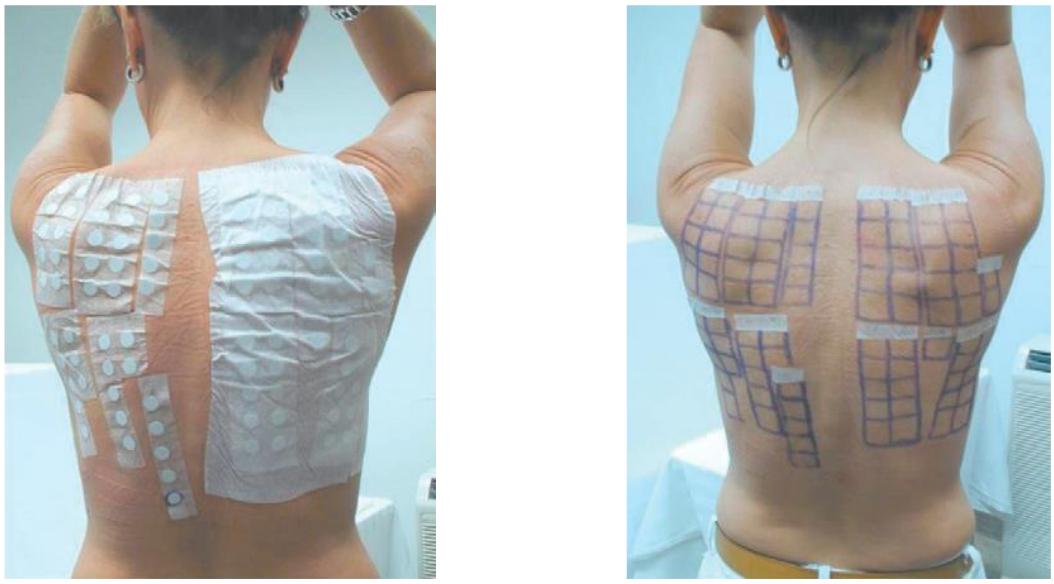

Fig. 10.4 The process of photopatch testing, where duplicate allergens are applied. One set is irradiated, while the other set remains covered (A). Results from both sets of allergens are read at the same time, at least two days following irradiation (B).

Once patch testing is complete, the clinician identifies the positive reactions and determines if the allergen is relevant to the dermatitis the patient is experiencing. For example, if a patient presents with

scalp, hairline, and facial dermatitis and has a positive reaction to para-phenylenediamine (PPD), this would be relevant because PPD is a component of permanent hair dyes and is a known cause of contact dermatitis. The patient should be questioned about exposures to hair dyes or other PPD-containing products, such as black henna tattoos. With a positive history of using products containing the allergen, there is a high likelihood that the specific allergen is causing the contact dermatitis.

Treatment and prevention

Treatment of contact dermatitis begins with identifying and avoiding the etiologic agent for the dermatitis. Patients should be given information detailing products that contain the allergen, as well as alternative products that are allergen free. Complete avoidance can be difficult, especially in the case of occupational exposure. If ICD is suspected, high-lipid creams, cotton gloves under occlusive gloves, and use of softened fabrics are techniques that can be utilized to minimize the dermatitis.

The most commonly used agents to treat contact dermatitis are corticosteroids. Topical steroids are applied twice per day, ranging from mild to super potent, depending on the location of the eruption and the age of the patient. In severe cases, systemic corticosteroids are used, such as prednisone 0.5–1.0 mg/kg per day. Therapy should be tapered over a sufficient amount of time, 2–3 weeks; to minimize the risk of a rebound flare. Adjunctive therapy includes Burow's and Domeboro's solution, oatmeal baths, and oral antihistamines. The most important aspect of treatment is patient counseling and avoidance of the allergen causing the contact dermatitis.