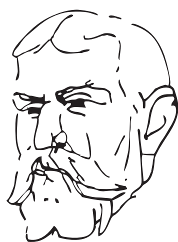
VOLUME LXXVI, ISSUE 1, JANUARY 2023

ISSN 0043-5147
E-ISSN 2719-342X

Wiadomości Lekarskie
Medical Advances

Official journal of Polish Medical Association has been published since 1928

INDEXED IN PUBMED/MEDLINE, SCOPUS, EMBASE, EBSCO, INDEX COPERNICUS,
POLISH MINISTRY OF EDUCATION AND SCIENCE, POLISH MEDICAL BIBLIOGRAPHY



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Medical Advances

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Grzegorz Sztank www.red-studio.eu

Publisher:
ALUNA Publishing House
ul. Przesmyckiego 29,
05-510 Konstancin – Jeziorna
www.wydawnictwo-aluna.pl
www.wiadomoscilekarskie.pl
www.wiadlek.pl

Yassir Alaa Muhammed Hassan Shubbar
CORRELATION BETWEEN DIFFERENT CLINICOPATHOLOGICAL PARAMETERS AND MOLECULAR SUBTYPES OF FEMALE BREAST CARCINOMA IN SOUTH REGION OF IRAQ 97

Nadiya O. Fedchyshyn, Vasyl Ya. Haida, Viktor Ye. Kavetskyi, Vadym Yu. Babii, Tetiana P. Husieva, Larysa Ya. Fedoniuk, Tetiana I. Pantiuk
FEATURES OF FORMING SELF-EDUCATIONAL COMPETENCE OF FUTURE DOCTORS 108

Tatyana M. Prozorova, Igor V. Zhulkevych, Serhiy M. Andreychyn, Neonila I. Korylchuk, Irina I. Hanberher, Svitlana S. Riabokon, Aleksander M. Kamyshnyi
EXPERIMENTAL GESTATIONAL DIABETES DISRUPTS THE FORMATION OF IMMUNE TOLERANCE IN OFFSPRING 115

Fadha Abdulameer Ghafil, Sahar A. Majeed, Heider Qassam, Haider W. Mardan, Najah R. Hadi
NEPHROPROTECTIVE EFFECT OF GAMMA-SECRETASE INHIBITOR ON SEPSIS- INDUCED RENAL INJURY IN MOUSE MODEL OF CLP 122

Hanna M. Kozhyna, Vsevolod V. Stebliuk, Yuliia O. Asieieva, Kateryna S. Zelenska, Kate V. Pronoza-Stebliuk
A COMPREHENSIVE APPROACH TO MEDICAL-PSYCHOLOGICAL SUPPORT FOR SERVICE WOMEN IN MODERN UKRAINE 131

Oleg. S. Chaban, Olena O. Khaustova, Dmytro O. Assonov, Lesia V. Sak
SAFETY AND EFFICACY OF THE COMPLEX DEPRILIUM® IN REDUCING SUBCLINICAL SYMPTOMS OF DEPRESSION IN PATIENTS WITH CHRONIC NON-COMMUNICABLE DISEASES: DOUBLE-BLIND RANDOMIZED CONTROLLED STUDY 136

Muhannad Mahmood Mohammed, Esraa K. Alnajim, Mohammed Abed Abdul Hussein, Najah R. Hadi
RISK FACTORS FOR DIABETIC NEPHROPATHY IN DIABETES MELLITUS TYPE 1 145

Khrystyna Pohranychna, Roman Ohonovskyi, Yuriy Rybert, Lidiya Minko, Oksana Hlova
EFFICACY OF ARTHROCENTESIS FOR TREATMENT OF INTERNAL POST-TRAUMATIC TEMPOROMANDIBULAR JOINT DISORDERS 155

Tamara Hristich, Dmytro Hontsariuk, Yana Teleki, Yuliya Serdulets, Evelina Zhygulova, Oksana Olinik, Oleh O. Ksenchyn
FEATURES OF THE CLINICAL COURSE OF OSTEOARTHRITIS IN COMBINATION WITH DIABETES MELLITUS 161

Ghada Hamid Naji, Worood Hameed Al-Zheery, Noor Yousif Fareed
DESIGN AND IN VITRO EVALUATION OF ACRIVASTINE AS ORODISPERSIBLE TABLET USING DIRECT COMPRESSION METHOD 170

Andriy Pidlisetskyy, Serhii Savosko, Igor Gayovich, Oleksii Dolhopolov, Volodymyr Biliavskyi
THE ULTRASONOGRAPHY EXAMINATION OF SKELETAL MUSCLES IN TRAUMATIC ISCHEMIA (EXPERIMENTAL STUDY) 175

Yurii O. Hrubar, Iryna Ya. Hrubar, Nadiia M. Hrabyk, Markiian Yu. Grubar, Yuliana Yu. Hrubar
INFLUENCE OF CRYOTHERAPY WITH PULSE COMPRESSION ON THE FUNCTIONAL CONDITION OF THE KNEE JOINT AFTER PARTIAL MENISCECTOMY 182

Oleksandr V. Tsyhykalo, Nataliia B. Kuzniak, Roman R. Dmytrenko, Pavlo P. Perebyjnis, Igor Yu. Oliinyk, Larysa Ya. Fedoniuk
FEATURES OF MORPHOGENESIS OF THE BONES OF THE HUMAN ORBIT 189

Jasim M. Salman, Jasim N. Al-Asadi, Husham H. Abdul-Ra'aoof, Jawad H. Ahmed, Ali H Reshak
COMPARISON OF INTRAMUSCULAR VERSUS INTRAVENOUS KETAMINE FOR SEDATION IN CHILDREN UNDERGOING MAGNETIC RESONANCE IMAGING EXAMINATION 198

ORIGINAL ARTICLE

EXPERIMENTAL GESTATIONAL DIABETES DISRUPTS THE FORMATION OF IMMUNE TOLERANCE IN OFFSPRING

DOI: 10.36740/WLek202301116

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ABSTRACT

The aim: To analyze the mRNA gene expression level of Aire, Deaf1, Foxp3, Ctla4, Il10, Nlrp3 and distribution of NLRP3+-cells in mesenteric lymph nodes (MLNs) of the offspring of rats with GD, both untreated and treated with glibenclamide and in conditions of insulin oral tolerance formation.

Materials and methods: The study involves 160 male rats, one- or six-month-old. The mRNA genes expression was studied by real time quantitative polymerase chain reaction. Structure of Nlrp3+ -cells population was studied by histological sections of MLNs.

Results: We observed AIRE gene repression, reduced mRNA levels of Deaf1 and the transcription factor Foxp3 in offspring of rats with GD. This was accompanied by inhibition of IL-10 gene expression and negative costimulatory molecules Ctla4. The development of the experimental GD was accompanied by transcriptional induction of the Nlrp3 gene in MLNs of descendants. The administration of glibenclamide to pregnant female rats with GD inhibited the transcription of the Nlrp3 gene only in one-month-old offspring (5.3-fold) and did not change it in six-month-old animals. In offspring of rats with GD, the density of the NLRP3+-lymphocyte population in the MLNs increased, more pronounced in one-month-old animals. The administration of glibenclamide to pregnant rats with GD reduced the number of NLRP3+ -lymphocytes only in one-month-old offspring (by 33.0 %), whereas this index in six month-old offspring even increased.

Conclusions: Experimental prenatal hyperglycemia leads to increased proinflammatory signaling and violation of peripheral immunological tolerance formation more pronounced at one month of life.

KEY WORDS: gene expression, insulin, mesenteric lymph nodes, glibenclamide, experimental gestational diabetes, NLRP3 - inflammasome

Wiad Lek. 2023;76(1):115-121

INTRODUCTION

Gestational diabetes (GD) – autoimmune disorder, caused by the destruction of β-cells of pancreatic islets by an immune-mediated process, has emerged as a global public health concern [1]. Formation of immunological tolerance to autoantigens is an important mechanism that prevents the development of autoimmune diseases (AIDs). Recently extrathymic expression in number of peripheral tissue -specific antigens (PTSAs), including such pancreatic antigens as insulin and proinsulin was found. Their ectopic transcription is regulated by autoimmune regulator (Aire) [2]. A lot of extrathymic Aire-expressing cells (eTACs) are found in lymphatic nodes and represent one of the critical factors of peripheral immunological tolerance (PIT) [3]. Stromal cells (fibroblast reticular cells, follicular dendritic cells and lymphatic endothelial cells) of mesenteric lymph nodes (MLNs) express PTSAs [4], but their expression is regulated not only by eTACs, but by the regulator of transcription – deformed autoregulatory factor 1 (Deaf1) [5]. Consequently, Aire and Deaf1 are important differentiation regulators of inducible regulatory T-cells (iTreg), which can express transcription factor Foxp3 [9, 10], their action realized through production of suppressor cytokines – IL10, IL13, IL35, TGFβ [6], perforin/granzyme-dependent cytolysis of effector cells and depends on the expression of negative costimulatory molecules such as cytotoxic T-lymphocyte-associated protein 4 (CTLA-4). Yang S. et al. demonstrated ability of Aire to generate in the prenatal period (up to 10 days after birth inclusive) special population of $FoxP3^{+}$Treg-cells, which remains stable in adults and mice [7].

GD can cause the immune disorders in offspring, because during pregnancy all immune mechanisms become activated [8]. Thus, Li Q. et al. demonstrated that interleukin-1β expression could be higher in offspring spleen cells when mother suffering from GD [9]. This phenomenon linked to the activation of NLRP3-inflammasome – multimeric protein belonging to the family

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of nod-like receptors, NLRs [10]. Glyburide, parthenolide and glibenclamide are proposed as medications, which have possibillity to change activity of NLRP3-inflammasome e.g. [11]. Glibenclamide is the most prominent, because it not only maintains the adequate glycemic control, but also could dicrease hyperglycemia-associated long-term outcomes in GD [12].

THE AIM

The aim of the current study was to analyze the mRNA gene expression level of *Aire*, *Deaf1, Foxp3, Ctla4*, *Il10, Nlrp3* and distribution of NLRP3+-cells in mesenteric lymph nodes (MLNs) of the offspring of rats with GD, both untreated and treated with glibenclamide and in conditions of insulin oral tolerance formation.

MATERIALS AND METHODS

The experimental animals, white Wistar male rats (n=160) were housed under standard conditions, with proper diet and water ad libitum at the animal facility of Zaporizhzhia State Medical University. Animal treatment and all experimental procedures were performed in compliance with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes. The study was approved by the Ethical Committee of Zaporizhzhia State Medical University.

Experimental study design comprised eight groups: one-month-old descendants of intact Wistar rats (group 1; n=20); six-month-old descendants of intact Wistar rats (group 2; n=20); onemonth-old descendants of Wistar rats with gestational diabetes (GD) (group 3; n=20); six-monthold descendants of Wistar rats with GD (group 4; n=20); one-month-old descendants of Wistar rats with GD, treated with insulin (group 5; n=20); six-month-old descendants of Wistar rats with GD, treated with insulin (group 6; n=20); one-month-old descendants of Wistar rats with GD treated with glibenclamide during pregnancy (group 7; n=20); six-month-old descendants of Wistar rats with GD treated with glibenclamide during pregnancy (group 8; n=20).

Experimental GD was induced by a single intraperitoneal administration of streptozotocin (STZ) (Sigma Chemical, USA) at a dose of 45 mg/kg body weight on the 15th day of pregnancy. Immediately prior to the administration, STZ was dissolved in 0.1 M citrate buffer (pH 4.5).

Blood glucose concentration was determined on the 3rd day after STZ administration using the glucose oxidase method with BIONIME Rightest TM GM 110 glucometer (Switzerland). Blood samples were taken from the tail vein. Animals with fasting glucose level of > 8.0 mmol/l were selected for study.

Glibenclamide («*Pharmak*», *Ukraine*) was administrated orally at a dose of 5 mg/kg for the 7 days to pregnant female rats after STZ administration.

Short-acting human insulin was administrate d orally using a pipette for the first 14 days of life (*ACTRAPID® HM, NOVO NORDISK, Denmark*) at a dose of 30 IU (1050 μg=1,05 mg, 1 IU corresponds to 35 μg of anhydrous human insulin).

MLNs of experimental animals were studied using real-time reverse transcription polymerase chain reaction (RT-PCR) techniques. Each rat was anaesthetized with ketamine hydrochloride at a dose of 100 mg/kg. An upper midline abdominal incision was made. All the MLNs identifiable along the line of the mesenteric blood vessels were carefully dissected off the mesentery.

Animal euthanasia was carried out by cardiac puncture under deep anaesthesia, in accordance with the requirements of the Animal Care Committee.

MLNs were placed in the Bouin's fluid, dehydrated with ethanol and embedded in paraffin. Molecular genetic studies were performed on archival material hold in biobank up to 2 years. RNA was extracted from 15 μm histological samples. They were dewaxed in xylene and rehydrated with descending concentrations of ethanol (100 %, 96 %, 70 %). Total RNA was obtained using of «Trizol *RNA* Prep 100» (*Isogen Lab LTD, Russia*), that contains *Trizol reagent* (lysis reagent, which includes denaturing agent guanidine thiocyanate and phenol with pH = 4.0) and *ExtraGene* E.

For obtaining cDNA and its reverse transcription RT-1 set «Syntol» (Russia) was used. The reaction mixture was taken in the volume of 25 μl containing 1 μl of random-6 primer, 2 μl total RNA, 8,5 μl deionized water, 12,5 μl 2,5x reaction mixture and 1 μl of reverse transcriptase MMLV-RT. Reverse transcription was conducted at 45°C for 45 min. Inactivation of MMLV-RT was achieved at 92°C for 5 min.

To determine the level of mRNA *Aire* (NM_001106379.1), *Deaf1* (NM_031801.1), *Foxp3* (NM_001108250.1), *IL10* (NM_012854.2), *Ctla4* (NM_031674.1) and *Nlrp3* (NM_001191642.1) we used thermocycler CFX96™Real-Time PCR Detection Systems («Bio-Rad Laboratories, Inc.», USA) with the set of reagents Maxima SYBR Green/ROX qPCR MasterMix (2X) (ThermoScientific, USA). The final reaction mixture for amplification includes coloring SYBR Green, Maxima HotStartTaq DNA Polymerase, 0,2 μl of forward and reverse specific primers, 1 μl cDNA. The reaction mixture brought to total volume 25 μl by adding deionized water. Specific primer pairs (5'-3') for analysis of target and reference genes were selected by the software PrimerBlast (www.ncbi.nlm.nih.gov/tools/primer-blast) and synthesized by Metabion (Germany) (Table I).

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Table I. List of primers used for real-time PCR

Gene	Primer	Tm,⁰C	Product length (bp)	Exon junction
Aire	F = GCCTAAAGCCAGTGATCCGA R = TCTCTACCCTGGGTTCCCTTT	59.82 59.85	43	850/851
Deaf1	F = GCAGAGAGGAAGGAGCAGTC R = GTGCACTCACTCATGGCCT	59.82 60	59	1605/1606
Foxp3	F = CGAGACTTGGAAGTCAGCCAC R = TCTGAGGCAGGCTGGATAACG	60.94 61.91	61	214/215
IL10	F=AGTGGAGCAGGTGAAGAATGA R=GACACCTTTGTCTTGGAGCTTATTA	59.02 59.06	49	445/446
Ctla4	F = TACAGTTTCCTGGTCACCGC R = AGGACTTCTTTTCTTTAGCGTCCT	59.97 59.96	57	567/568
Nlrp3	F = AGCTAAGAAGGACCAGCCAG R = CGTGCATGCATCATTCCACTC	59 60	40	713/714
GAPDH	F = GCCTGGAGAAACCTGCCAAG R =GCCTGCTTCACCACCTTCT	61 60	52	825/826

Table II. Normalized relative quantity of mRNA Aire, Deaf1 and Foxp3 genes in MLN cells

Target	Sample	Expression Fold Change	Fold Regulations	P
AIRE	gd1 vs c1	0,12	-8.1	< 0.05
Deaf1	gd1 vs c1	1,20	1,20	
Foxp3	gd1 vs c1	0,02	-50,0	< 0.05
AIRE	gd6 vs c6	0,44	-2.3	< 0.05
Deaf1	gd6 vs c6	0,11	-9,2	< 0.05
Foxp3	gd6 vs c6	0,39	-2.5	< 0.05
AIRE	gd1+ins vs gd1	13,2	13,2	< 0.05
Deaf1	gd1+ins vs gd1	11,5	11,5	< 0.05
Foxp3	gd1+ins vs gd1	5,2	5,2	< 0.05
AIRE	gd6+ins vs gd6	2,0	2,0	< 0.05
Deaf1	gd6+ins vs gd6	1,2	1,2	
Foxp3	gd6+ins vs gd6	3,3	3,3	< 0.05

*Fold-Change (2^(-Delta Delta CT)) is the normalized gene expression (2^(- Delta CT)) in the test sample (gd1, gd6, gd1+ins, gd6+ins) divided the normalized gene expression (2^(- Delta CT)) in the control sample. Fold-Regulation represents fold-change results in a biologically meaningful way. Normalized to reference gene GAPDH by the method ΔΔCt. c1, c6 – control 1 and 6 months; gd1, gd6 – offspring of the experimental GD rats; gd1+ins, gd6+ins – after insulin administrations.

After initial denaturation at 95°C for 10 min amplification was implemented in 45 cycles including following stages: denaturation – 95°C for 15 sec., annealing at 59–61°C for 30–60 sec., elongation at 72°C for 30 sec. [13].

The reference gene was glyceraldehyde 3-phosphate dehydrogenase *(GAPDH)* gene. Normalized relative quantity of cDNA target genes was determined by the method ΔΔCt. Statistical data analysis of PCR were conducted using available software CFX Manager ™(Bio-Rad, USA). Experiment included negative controls: no template controls (cDNA and mRNA) and no reverse transcriptase control. All amplification reactions were performed on individual samples three times.

Structure of $Nlrp3^{+}$-cells population was analyzed in the serial histological sections of MLN. Serial sections of 5 µm thick were made on a rotary microtome MICROM HR-360 (Microm, Germany), then they were dewaxed in xylene and rehydrated with descending concentrations of ethanol (100 %, 96 %, 70 %), washed with 0.1 M phosphate buffer (pH=7,4) and colored with Nlrp3 rabbit polyclonal antibodies (Cryopyrin, H-66) (Santa Cruz Biotechnology, USA, sc-66846) for 18 hours in a humid chamber at T=4 °C. After washing with 0.1 M phosphate buffer, samples were incubated for 60 min at T=37 °C with secondary antibody solution to rabbit IgG (Santa Cruz Biotechnology, USA), conjugated with FITC. After incubation, all sections were washed with 0.1 M phosphate buffer and placed in a mixture of glycerol phosphate buffer (1:9) for subsequent fluorescent microscopy. Histological sections were studied with the

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software Image J (NIH, USA), than the morphometric and densitometric characteristics of immunopositive cells and were measured. We determined the absolute (number of cells per 1 mm^2) and relative (%) density of different subsets of $Nlrp3^+$-lymphocytes in cortex and medullary cords of MLNs.

STATISTICAL ANALYSIS

The experimental data were processed and analysed using the software STATISTICA 6.0 (StatSoftInc., №AXXR712D833214FAN5, USA). The distribution of data was analyzed by Kolmogorov -Smirnov criterion. The obtained values had a normal distribution, so the difference between the groups was analyzed using the Student's t-criterion. All data were presented as M (mean) ± m (standard error). A probability level (p value) of less than 0.05 was considered to be statistically significant.

RESULTS

Investigation of *Aire* gene expression in MLNs showed that in offspring of rats with GD there is a significant reduction of mRNA of autoimmune regulator by 8.1 times ($p<0.05$) in one month-old (group 3) and by 2.3 times ($p<0.05$) in six-month-old animals (group 4) vs group 1 and 2 (Table II). mRNA content of transcription regulator *Deaf1* in one-month-old animals did not changed significantly, and in six-month-old descendants we observed its reduction by 9.2 times ($p<0.05$) vs group 1 and 2 (Table II). As for mRNA of transcription factor *Foxp3*, there was revealed a significant decrease by 50.0 times ($p<0.05$) in one-month-old rats (group 3), and by 2.5 times ($p<0.05$) in the six-month-old animals (group 4) vs group 1 and 2 (Table II).

Offspring of rats with GD, that were administered orally insulin during 14 first days of life, showed increasing of *Aire* gene transcriptional induction mostly in one-month-old animals (group 5) – the level of mRNA has increased by 13.2 times ($p<0.05$) vs group 3. In six-month-old animals (group 6) this index increased by 2.0 times ($p<0.05$) vs group 4 (Table II). Transcription regulator *Deaf1* in one-month-old animals (group 5) showed a significant increase by 11.5 times ($p<0.05$) vs group 3, and in six-month-old animals (group 6) it was similar to this index in group 4 (Table II).

Studies have shown that expression of the transcription factor *Foxp3* in one-month-old rats was increase of *Foxp3* mRNA by 5.2 times ($p<0.05$) vs group 3, in the six-month-old animals rise was 3.3 fold ($p<0.05$) vs group 4 (Table II).

In experimental groups 5 and 6 (one- and six-month-old offspring of rats with GD that received orally insulin) mRNA expression of costimulatory molecules *Ctla4* and Treg-dependent suppressor cytokine *IL-10* has also been investigated. We have found that relative quantity of *Ctla4* mRNA gene increased by 12.2 times ($p<0.05$) in one-month-old animals vs group 3. In six-monthold rats this index significantly did not changed vs group 4. Contents of mRNA *IL10*, on the contrary, in one-month-old animals was unaltered vs group 3, but in six-month-old rats it increased by 15.0 times ($p<0.05$) vs group 4.

Investigation of *Nlrp3* gene expression in MLNs showed that in the offspring of rats with GD there was a significant (5-fold) increasing of mRNA of this protein in one-month-old rats ($p<0.05$) and 3-fold increasing ($p<0.05$) in six-month-old animals vs group 1 and 2. In rats of group 7 and 8 (one- and six-month-old offspring of animals with GD that received glibenclamide during pregnancy) we have found a significant (by 5.3 times) decrease of *Nlrp* gene expression ($p<0.05$) in one-month-old, and absence of significant changes in six-month-old animals.

Studying the distribution of specific subpopulations of $Nlrp3^+$-cells we have found that total density of immunopositive cells in MLNs cortical plateau of one-month-old offspring of animals with GD increased by 49.0 % ($p<0.05$) vs group 1. In six-month-old animals comparative analysis revealed no significant changes vs group 2. Total number of $Nlrp3^+$-cells in MLNs medullary cords of one-month-old offspring of animals with GD was significantly increased by 44.0 % ($p<0.05$) vs group 1. The study of materials taken from the six-month-old rats showed an increase in the total density by 69.0 % ($p<0.05$) vs group 2. Analysis of MLNs sections in the experimental GD offspring of rats treated with glibenclamide during pregnancy have showed that in cortical plateau of MLNs in one-month-old animals we obtained reducing of the total number of $Nlrp3^+$-cells by 33.0 % ($p<0.05$) vs group 3. In six-month-old rats there were not significant changes in the number of immunopositive cells vs group 4. Total density of $Nlrp3^+$-cells in one-month-old animals did not changed significantly and in six-month-old rats it increased by 29.0 % ($p<0.05$).

DISCUSSION

The modern search for effective targeted therapy [13] for endocrine diseases is based on transcriptome [14], variome [15-16], and proteome data [17-19]. Peyer's patches (PP) and mesenteric lymph nodes (MLNs), which are present in the wall of the intestinal tube are the main components for for immune responses, they play an important role in the mechanisms of preventing the active immune response against usually harmless environmental antigens [20]. PP and MLNs considered

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to be the principal site for the induction of oral tolerance (OT) preventing immune response to an orally administered antigen. MLNs have distinctions from PP and peripheral lymph nodes and serve as a crossroads between the peripheral and mucosal recirculation pathways for antigens [21]. Such antigen recirculation occurs from the lamina propria into the MLNs and mediated by $CD103^+$ dendritic cells (DCs) and was found for OT systemic effect [21].

On the other hand, clinical manifestation of T1DM is preceded by the development of autoantibodies to different islet autoantigens, marking the loss of immunological tolerance to β cells. Most trials attempting immune intervention have been conducted in patients with recent onset T1DM (usually within 6 weeks of diagnosis), and have had varying but only limited success. This outcome might partly result from the stage of disease and progressive loss of β cells, in addition to the burden of poor glycaemic control and metabolic β-cell stress over and above the inflammatory insult. Unfortunately, the few attempts to prevent T1DM using immunotherapy in seropositive individuals at risk of the disease were unsuccessful. Bonifacio E. et al. demonstrated that oral administration of 67.5 mg of insulin, compared with placebo, resulted in an immune response without hypoglycemia, allergic and autoimmune reactions [22].

The inflammasomes and the complement system are traditionally viewed as quintessential components of innate immunity required for the detection and elimination of pathogens. But a direct role for NLRP3 in human adaptive immune cells has not been described yet. In recent years, data suggested that NLRP3 could be expressed by mouse and human lymphocytes and has an ability to adjust the differentiation of Th1, Th2 Th17-cells. Recently, Arbore G. et al. have shown that NLRP3 inflammasome assembles in human CD4+ T-cells and initiates caspase-1–dependent interleukin-1β secretion, thereby promoting interferon-γ production and T-helper 1 (TH1) differentiation in an autocrine fashion [23]. Furthermore, Bruchard M. et al. recently showed the ability of NLRP3 to act as a key transcription factor that controls the Th2-differentiation [24]. In Th2 cells NLRP3 binds to promoter IL4 and activates it in conjunction with transcription factor IRF4. In contrast to Th1, where NLRP3 is detected mainly in the cytoplasm by methods of immunofluorescence microscopy, in the Th2-cells it is localized mainly in the nucleus. It is possible that such a nuclear localization function can promote inflammasome transcription. This work showed that NLRP3 should be seen not only as a key inflammasome component, but as a transcription factor in cells $CD4^+$ Th2 [24]. Finally, the mechanisms of IL-1β-induced Th17 differentiation are related to the ability of TGF-β to induce expression RORγt in naive T-cells [25]. Studies in vitro have shown that IL-1β induces the expression of IRF-4, positively regulates IL-21-mediated expression of transcription factors STAT-3 and RORγt [25]. At the same time, NLRP3-inflammasome is one of the sencetive indices of metabolic stress developing diabetes [26-28]. NLRP3--deficient NOD-mice are protected from developing diabetes by reducing migration of diabetogenic lymphocytes in the pancreatic islets.

NLRP3-inflammasome is an important pharmacological target for blocking a number of diabetes complications [29], and the ability of glibenclamide to inhibit the formation of NLRP3 can affect the risk of inflammatory and AIDs in the offspring of mothers with GD. Recent research by Lamprianou S. et al. demonstrated that glibenclamide protects NOD mice from progressing hyperglycemia and loss of insulin-producing β-cells [30]. Although the administration of glibenclamide did not stop the development of insulitis, but induced a shift of the phenotype of immune cells and protects cells of insulinoma MIN6 from apoptosis and loss of connexin Cx36 [30].

CONCLUSIONS

1. The investigation of transcriptional activity of genes-regulators of the peripheral immunological tolerance formation in MLNs of the offspring of rats with GD showed the repression of *Aire* and *Deaf1* mRNA. These changes violate ectopic transcription of pancreatic antigens in MLNs. Reduction of mRNA *Foxp3* level leads to a deficiency of suppressor signaling, which is confirmed by inhibition of suppressor cytokine *IL10* gene expression and negative costimulatory molecules *Ctla4*. Oral administration of insulin during the first 14 days of life stopped these changes, causing transcription activation of *AIRE, Deaf1, Foxp3, Ctla4* and *Il10* genes.
2. The development of the experimental GD is accompanied by transcriptional induction of the *Nlrp3* gene in MLNs of descendants, whose mRNA level increased 5-fold ($p<0.05$) in onemonth-old and 3-fold ($p<0.05$) in six-month-old animals. The administration of glibenclamide to pregnant rats with GD inhibited the transcription of the *Nlrp3* gene only in one-month-old offspring (by 5.3 times, $p<0.05$) and did not change it in the group of six-month-old animals.
3. In the offspring of rats with GD, the density of the NLRP3+-lymphocyte population in the MLNs increased, more pronounced in one-month-old animals. The administration of glibenclamide to pregnant rats with GD reduced the number of $NLRP3^+$-lymphocytes only in one-month-old offspring, whereas this index in six-month-old offspring even increased.

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REFERENCES

1. Eades C.E., Cameron D.M., Evans J.M. Prevalence of gestational diabetes mellitus in Europe: A meta-analysis. Diabetes Res Clin Pract. 2017;129:173-181. doi: 10.1016/j.diabres.2017.03.030.
2. Metzger T., Anderson M. Control of central and peripheral tolerance by Aire. Immunol Rev. 2011;241(1):89-103. doi: 10.1111/j.1600-065X.2011.01008.x.
3. Carpino G., Puca R., Cardinale V. et al. Peribiliary glands as a niche of extrapancreatic precursors yielding insulin-producing cells in experimental and human diabetes stem. Cells. 2016;34(5):1332-1342. doi: 10.1002/stem.2311.
4. Cohen J., Tewalt E., Rouhani S. Tolerogenic properties of lymphatic endothelial cells are controlled by the lymph node microenvironment. PLoS ONE. 2014;9(2):e87740. doi: 10.1371/journal.pone.0087740.
5. Yip L., Fuhlbrigge R., Taylor C. et al. Inflammation and hyperglycemia mediate Deaf1 splicing in the pancreatic lymph nodes via distinct pathways during type 1 diabetes. Diabetes. 2015;64(2):604-617. doi: 10.2337/db14-0803.
6. Wang S., Gao X., Shen G. et al. Interleukin-10 deficiency impairs regulatory T cell-derived neuropilin-1 functions and promotes Th1 and Th17 immunity. Sci Rep. 2016;14(6):242-249. doi: 10.1038/srep24249.
7. Yang S., Fujikado N., Kolodin D. et al. Immune tolerance. Regulatory T cells generated early in life play a distinct role in maintaining self-tolerance. Science. 2015;348(6234):589-594. doi: 10.1126/science.aaa7017.
8. Wojcik M., Zieleniak A., Zurawska-Klis M. et al. Increased expression of immune-related genes in leukocytes of patients with diagnosed gestational diabetes mellitus (GDM). Exp Biol Med (Maywood). 2016;241(5):457-465. doi: 10.1177/1535370215615699.
9. Li Q., Pereira T., Moyce B. et al. In utero exposure to gestational diabetes mellitus conditions TLR4 and TLR2 activated IL-1beta responses in spleen cells from rat offspring. Biochim Biophys Acta. 2016;1862(11):2137-2146. doi: 10.1016/j.bbadis.2016.08.004.
10. Jo E.K., Kim J.K., Shin D.M., Sasakawa C. Molecular mechanisms regulating NLRP3 inflammasome activation. Cell Mol Immunol. 2016;13(2):148-159. doi: 10.1038/cmi.2015.95.
11. Coll R.C., Robertson A.A., Chae J.J. et al. A small-molecule inhibitor of the NLRP3 inflammasome for the treatment of inflammatory diseases. Nat Med. 2015;21:248-255. doi: 10.1038/nm.3806.
12. Koren R., Ashwal E., Hod M., Toledano Y. Insulin detemir versus glyburide in women with gestational diabetes mellitus. Gynecol Endocrinol. 2016;32(11):916-919. doi: 10.1080/09513590.2016.1209479.
13. Nosulenko I.S., Voskoboynik O.Y., Berest G.G. et al. Synthesis and Antimicrobial Activity of 6-Thioxo-6,7-dihydro-2H-[1,2,4]triazino[2,3-c]-quinazolin-2-one Derivatives. Sci Pharm. 2014;82(3):483-500. doi:10.3797/scipharm.1402-10.
14. Prozorova T.M., Kamyshna V.A., Kamyshny O.M. Effect of experimental gestational diabetes and administration of glibenclamide on mRNA level of NLRP3-inflammasome and distribution of NLRP3+-cells in mesenteric lymph nodes in progeny. Pathologia. 2017;14(2):149-155. doi: 10.14739/2310-1237.2017.2.109269.
15. Dzhuryak V., Sydorchuk L., Sydorchuk A. et al. The cytochrome 11B2 aldosterone synthase gene CYP11B2 (RS1799998) polymorphism associates with chronic kidney disease in hypertensive patients. Biointerface Res Appl Chem. 2020;10(3):5406-11. doi: 10.33263/BRIAC103.406411.
16. Lyubomirskaya E.S., Kamyshnyi A.M., Krut Y.Y. et al. SNPs and transcriptional activity of genes of innate and adaptive immunity at the maternal-fetal interface in woman with preterm labour, associated with preterm premature rupture of membranes. Wiad Lek. 2020;73(1):25-30.
17. Topol I.A., Kamyshny A.M., Abramov A.V., Kolesnik Y.M. Expression of XBP1 in lymphocytes of the small intestine in rats under chronic social stress and modulation of intestinal microflora composition. Fiziol Zh. 2014;60(2):38-44.
18. Topol I., Kamyshny A. Study of expression of TLR2, TLR4 and transckription factor NF-kB structures of galt of rats in the conditions of the chronic social stress and modulation of structure of intestinal microflora. Georgian Med News. 2013;(225):115-22.
19. Degen A.S., Krynytska I.Y., Kamyshnyi A.M. Changes in the transcriptional activity of the entero-insular axis genes in streptozotocin-induced diabetes and after the administration of TNF-α non-selective blockers. Endocr Regul. 2020;54(3):160-171. doi: 10.2478/enr-2020-0019.
20. Agace W.W. T-cell recruitment to the intestinal mucosa. Trends Immunol. 2008;29(11):514-522. doi: 10.1016/j.it.2008.08.003.
21. Kunkel D., Kirchhoff D., Nishikawa S-I. et al. Visualization of peptide presentation following oral application of antigen in normal and Peyer's patches-deficient mice. Eur J Immunol. 2003;33(5):1292-1301. doi: 10.1002/eji.200323383.
22. Bonifacio E., Ziegler A.G., Klingensmith G. et al. Effects of high-dose oral insulin on immune responses in children at high risk for type 1 diabetes: The Pre-POINT randomized clinical trial. JAMA. 2015;313(15):1541-1549. doi: 10.1001/jama.2015.2928.
23. Arbore G., West E.E., Spolski R. T helper 1 immunity requires complement-driven NLRP3inflammasome activity in $CD4^{+}$ T-cells. Science. 2016;352(6292):aad1210. doi: 10.1126/science.aad1210.
24. Bruchard M., Rebé C., Derangère V. The receptor NLRP3 is a transcriptional regulator of TH2 differentiation. Nat Immunol. 2015;16(8):859-870. doi: 10.1038/ni.3202.
25. Mailer R.K., Joly A.L., Liu S. et al. IL-1beta promotes Th17 differentiation by inducing alternative splicing of FOXP3. Sci Rep. 2015;5:146-174. doi: 10.1038/srep14674.

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26. Prozorova T., Tokarskyy O., Fedoniuk L. et al. Changes in the transcriptional activity of the lymphocyte homing regulatory genes Madcam1, Cxcr3, Ccr7 and S1pr1 affect structure of the population of T-bet+, Rorγt+ and Foxp3+ cells in mesenteric lymph nodes in offspring of rats with experimental gestational diabetes. Rom J Diabetes Nutr Metab Dis. 2020;27(3):185-194. doi: 10.46389/rjd-2020-1029.
27. Putilin D., Kamyshnyi A. Changes of Glut1, mTOR and AMPK1α gene expression in pancreatic lymph node lymphocytes of rats with experimental diabetes mellitus. Medical Immunology (Russia). 2016; 18 (4): 339-346. doi: 10.15789/1563-0625-2016-4-339-346.
28. Putilin D.A., Evchenko S.Yu., Fedoniuk L.Ya. et al. The influence of metformin to the transcriptional activity of the mTOR and FOX3 genes in parapancreatic adipose tissue of streptozotocin-induced diabetic rats. J. Med. Life. 2020;13(1):50-55. doi: 10.25122/jml-2020-0029.
29. Zherebiatiev A., Kamyshnyi A. Expression Levels of Proinflammatory Cytokines and NLRP3 Inflammasome in an Experimental Model of Oxazolone-induced Colitis. Iran J Allergy Asthma Immunol. 2016;15(1):39-45.
30. Lamprianou S., Gysemans C., Bou Saab J. et al. Glibenclamide Prevents Diabetes in NOD Mice. PLoS One. 2016;11(12):e0168839. doi:10.1371/journal.pone.0168839.

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Conflict of interest:
The Authors declare no conflict of interest.

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Received: 24.11.2021
Accepted: 14.11.2022

A - Work concept and design, **B** – Data collection and analysis, **C** – Responsibility for statistical analysis, **D** – Writing the article, **E** – Critical review, **F** – Final approval of the article

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