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ORIGINAL ARTICLE

Luminescence of blood leukocytes fluorochromated with acridine orange of operated patients in the dynamics of emotional, anesthetic, and surgical stress

E. Fedotov[1], K. Aleksandrova[1], D. Vasylyev[1], O. Frolov[2], J. Vavrysevych[3], B. Gutyj[3*], O. Yaremko[3], N. Magrelo[3], H. Sus[3], U.Vus[3]

[1]Zaporizhzhia State Medical University, Zaporizhzhia, Ukraine
[2]Zaporizhzhia National University, Zaporizhzhia, Ukraine
[3]Stepan Gzhytskyi National University of Veterinary Medicine and Biotechologies Lviv, Ukraine
**Corresponding author E-mail: bvh@ukr.net*
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The dynamics of luminescence indices of blood leukocytes, fluorochromated with acridine orange, were studied in operated patients. The most informative of them was the luminescence intensity at 640 nm to characterize the state of immunity. It radically increased during the traumatic periods of the operation and during the activation of reparative processes. Significant radical changes of I = 640 nm in blood lymphocytes, which occurred during the traumatic period of the operation, persisted for a week after the operation, indicating the degree of participation of the immune system in reparative regeneration.
Keywords: acridine orange; leukocyte; stress, 640 nm

Introduction

The immune and neuroendocrine systems are functionally interconnected by typical receptors, mediators, and target cells due to their impact on homeostasis regulation (Haitov & Pinegii, 1998). However, the participation of the immune system in the general adaptation syndrome has been shown mainly in experimental studies, while in humans under various stressful situations, this problem has been poorly studied. Under these circumstances, acridine orange (AO) – the dual-mode luminophore, is promising, which selectively reacts with nucleic acids (NA) in fixed cells at pH 4.2-6.0 (Karnauhova et al., 2008; Amado et al., 2017; Damas-Souza et al., 2019; Byvaltsev et al., 2019). Moreover, its monomers associate with double-stranded nucleic acids, and the maximum luminous intensity lies in the green region of the spectrum (530 nm). In comparison, the dimeric form of AO combines with single-stranded nucleic acids (mainly RNA and metabolically active DNA regions) and is characterized by red luminescence with a maximum emission at 640 nm. Therefore, the ratio of single- and double-stranded nucleic acids can be used to assess the rate of the protein synthesis and, consequently, its functional state.

Aims and scope. To study the features of the luminescence of functionally heterogeneous circulating blood leukocytes at different stages of surgical treatment, under various stress effects (emotional, anesthetic, surgical) and periods of expressed immunity associated with intensive repair processes.

Materials and methods

Venous blood was collected from 25 individuals aged between 28 to 58 years. All of them underwent planned surgical intervention for the tumor process of the central nervous system.

We used types of anesthesia similar to their antistress mechanism: ataralgesia (8 people), neuroleptanalgesia (9 people), propofol with fentanyl (8 people). Blood sampling was carried out in several stages: 1) before the operation; 2) after 30-40 minutes after premedication; 3) after 30-40 minutes of induction of anesthesia and intubation; 4) after 30-40 minutes before the operation starts (traumatic stage of the operation); 5) at the end of the operation; 6) one day after the operation; 7) one week after surgery.

Luminescence smears were prepared from leucocyte concentrate obtained by precipitation of erythrocytes with gelatin. In this case, to avoid nonspecific luminescence, autoplasma was replaced with fetal calf serum, the spontaneous luminescence of which was at a minimum level. The smears were fixed in a mixture of acetone and absolute ethyl alcohol in ratio 1:1, then went through a battery of alcohols with a lowering concentration, fluorochromated with AO solution at a concentration of 1: 25000

in citrate-phosphate buffer pH 6.0, washed from unreacted fluorochrome in the same buffer, applied 2-3 drops of AO protector from photodestruction (Damas-Souza et al., 2019) were covered with a glass and diluted in 30% polystyrene solution in xylene. The algorithm studied the luminescence of 50 lymphocytes and 50 segmented neutrophils for analyzing blood counts in smears using a microspectrofluorimeter. To exclude the influence of technical variations of the method on the luminescence indices, the preparations of all stages were fluorochromated and analyzed simultaneously. The luminescence intensity (I) was studied at the maximum light wavelengths of 530 nm (I = 530 nm) and 640 nm (I = 640 nm); the luminescence excitation spectrum was 436 nm. The A-parameter was calculated from the ratio of I = 640 nm to I = 530 nm (Damas-Souza et al., 2019).

Results and discussion

According to Table 1, the control stage showed no apparent violations of the leukocyte formula. Induction and patient transfer of ALV were exhibited a decrease of all types of white blood cells (monocyte number remained unchanged). The onset of the anxiety phase characterizes the redistribution of white blood cells at this stage during the development of general adaptation syndrome (GAS), according to Hans Selye (Savvina et al., 2017; Dzhumabekov et al., 2018).
Total leucocyte count, starting from the 4th stage, increased up to the 6th stage (a day after the operation), at which it reached a maximum (188.68%). Our attention is drawn to a dramatic increase in the number of leukocytes by the end of the operation compared with the previous stage (11.73 ± 0.98 and 7.23 ± 0.65 respectively), which is explained by an increase in the antigenic load as a result of the surgical trauma. The development of leukocytosis during the checkup occurred mainly due to neutrophilia.

Table 1. Blood formula of surgical patients (CNS tumors) during checkup stages

Stage	WBC number, g/l	E,%	Hemogram				
			N			L	
			B, %	S, %	M, %	%	g/l
1	7.07±0.51	2.64±0.20	4.22±0.22	61.61±2.19	4.61±0.39	26.92±1.86	1.92±0.13
2	7.09±0.71	2.36±0.25	5.23±0.25	62.57±1.76	4.64±0.41	25.27±1.46	1.79±0.16
3	6.16±0.60	2.63±0.15	3.22±0.27	60.84±1.43	4.84±0.42	28.5±1.37	1.77±0.14
4	7.23±0.65	2.21±0.17	4.26±0.33	61.53±3.54	3.89±0.25	27.05±2.68	1.82±0.15
5	11.73±0.98	1.74±0.13	5.34±0.35	70.97±2.82	3.47±0.21	19.92±1.51	2.13±0.21
6	13.34±1.13	1.24±0.10	5.95±0.41	70±2.91	3.53±0.24	19.26±1.03	2.65±0.17
7	12.02±1.18	2.15±0.18	3.62±0.24	68.69±3.49	4.12±0.32	21.46±1.63	2.44±0.15

Table 2. WBC luminescence of surgical patients (CNS tumors) during checkup stages

Indices	Stages						
	1	2	3	4	5	6	7
I_{530} l. b.	1.46±0.11	1.87±0.13	1.55±0.09	1.48±0.10	1.75±0.11	1.24±0.08	1.62±0.09
I_{640} l. b.	2.18±0.12	2.65±0.14	2.46±0.12	2.25±0.12	2.68±0.14	1.80±0.11	2.57±0.13
A-par., l.b.	0.45±0.03	0.39±0.03	0.49±0.04	0.74±0.05	0.87±0.05	0.80±0.04	0.96±0.05
LI_{640}, nm, l.	0.33±0.02	0.30±0.02	0.37±0.03	0.66±0.03	0.62±0.03	0.56±0.02	0.59±0.02

Note: l. – lymphocytes, b – band leukocytes, A-par. – A-parameter, LI_{640} – luminescence intensity at 640 nm

The dynamics of the WBC luminescence of the examined individuals are presented in Table 2. The neutrophils luminescence indices at 530 nm were significantly higher than in lymphocytes at all checkup stages, which coincides with the literature data (Nahirnyi & Kozak, 2015; Popko & Dmytriiev, 2017) and with the statement that neutrophils entering the general recirculation are on the final stage of differentiation, when their nuclear chromatin is inactive and strongly condensed, which leads to an increase in the proportion of double-stranded nucleic acids. The absence of statistically significant shifts for lymphocytes at I = 530 nm during the checkup indicates the metabolic inertness of their nuclei because lymphocytes are recirculated in the G0 phase of the mitotic cycle. However, the metabolic inertness of the nucleus for most of them, despite neutrophils, is temporary. After receiving mitogenic and antigenic signals, they re-enter the mitotic cycle in the lymphoid organs and undergo certain stages of immunogenesis, forming clones of committed, sensitized lymphocytes and memory cells. Specialized lymphocytes enter the general recirculation. In such a stage, only accumulated RNAs can prove the antecedent metabolic activity of their nucleus. Blood lymphocytes are heterogeneous in immunological activity, which coincides with the variation of I = 640 nm.

Consequently, the number of single-stranded RNAs in lymphocytes, which we register at I = 640 nm, can be considered a residual sign of their previous immunogenesis, and the functional state of the immune system at the time of examination can be assessed by the level of cell luminescence of their spectrum. The A-parameter, used by other researchers, repeats the variations in circulating lymphocytes at I = 640 nm. Therefore, it is not very suitable for their functional characteristics.

This conclusion is fully confirmed by I = 640 nm dynamics in different stages of examination of operated patients. Moreover, the nature of this variation reflected the participation of the immune system in the general adaptation syndrome to stress effects, which are preoperative preparation, intubation, and surgical trauma, which is an additional argument in favor of the functional relationship of these homeostatic systems. So, I = 640 nm in 30-40 minutes after standard premedication decreased compared to the initial level. The probable cause for this decrease is the inhibition of activated lymphocytes in the parenchymal organs under the influence of glucocorticoid hormones released into the blood by the adrenal glands in the body's anxiety against a stressor (psychogenic stress). By the third stage of the examination, I = 640 nm returned to its initial level, which can be regarded as the return of activated lymphocytes to the general recirculation resulting from an increase in the body's resistance and the positive effect general anesthesia.

The operation is intense stress for the body, accompanied by neurovegetative reactions, the ingress of mediators, cytokines into the bloodstream, which activate the immune system. In addition, during this period, the homeostasis of antigens is disrupted, and detritus autoantigens are released, which activates the immune system's morphogenetic (reparative) reactions. We registered functional changes in the immune system by the abrupt change in I = 640 nm after the start of the operation (stage 4). By the end of the operation, I = 640 nm increased further to its maximum values, reflecting the high degree of stress of the immune system to anesthetic and surgical stress. It should be noted that an almost twofold increase in I = 640 nm during the traumatic period of the operation is due not only to an increase in the luminescence of individual lymphocytes but also to an increase in the proportion of cells with a high level of I = 640 nm in recirculation.

The latter fact can be explained from the standpoint of polyclonal activation of lymphocytes by those mentioned above autoantigenic and mitogenic factors, which appear in large numbers and diversity in the liquid medium of the body. Considering the phylogenetic dependence of this reaction, it can be viewed as a protective one aimed at detoxification and restoration of homeostasis of antigens. However, when the threshold of expediency in magnitude and duration is exceeded, the polyclonal reaction of lymphocytes can cause secondary immunodeficiency, since the lymphocytes involved in a specific immunogenesis no longer respond to other antigenic and mitogenic stimuli. Secondary immunodeficiency often accompanies surgical treatment and is the leading cause of its operational complications (Dzhumabekov et al., 2018).

The next day after the operation and seven days after it, the lymphocyte I = 640 nm remains high and already characterizes the mobilization of the immune system for reparative processes. A feature of the last stage of the survey was the multidirectionality of I = 640 nm. In some patients, it continued to increase; in others, it decreased to the initial values, which was accompanied by the range of variation in the indicator and an increase in the standard deviation. These differences have probably characterized the participation of the immune system in the regulation of reparative morphogenesis and the degree of its completion.

The greater set of I = 640 nm in comparison with another I = 530 nm for characteristics of the functional activity of lymphocytes is confirmed by the data of the correlative analysis on the relationship of the indicated luminescence indices with the A-parameter. So at the 1st and especially at the 2nd stages of the checkup, when the number of activated lymphocytes in the blood is small, and low I = 530 nm shows a weak connection with the A-parameter ($r = 0.13$; $r = 0.09$, $P>0.05$, stages 1 and 2, respectively). Starting from the third stage of the survey, with an increase in I = 640 nm, the reliable relationship with the A-parameter from the mean value ($r = 0.37$; 0.38; 0.39, $P <0.05$, respectively at 3, 4, 5 stages) increased to a high ($r = 0.71$; 0.78, $P <0.01$, respectively at 6 and 7 stages). The negative correlation of I = 530 nm with the A-parameter at all periods of the examination, without a definite regularity, ranged from 0.51 to 0.56, $P <0.05$.

The dynamics in different checkup stages of I = 640 nm of neutrophils repeated that of lymphocytes ($r = 0.83$, $P <0.001$), but was significantly less in amplitude, which is due to their unequal role in immunological reactions: neutrophils use the substances from granules accumulated at the stages of their histogenesis, while lymphocytes synthesize immune factors *de novo* during immunogenesis. At the same time, an increase of I = 640 nm in segmented neutrophils with reinforcement of immune reactions can be interpreted as an indicator of a shift in the blood formula to the left, which means an exit into the general recirculation of cells with varying degrees of differentiation. Morphologically, this shift is manifested by the appearance in the blood of young and stab neutrophils. However, we studied luminescence in segmented neutrophils; therefore, the different level of I = 640 nm in a morphologically homogeneous group of neutrophils indicates their differences of differentiation and may become an additional sign of the activity the neutrophil reaction (Fig. 1).

A similar shift to the left took place during lymphocyte histogenesis. We observed in the operational and postoperative periods large and medium lymphocytes with blast-like signs, accompanied by a high level of I = 640 nm. Such lymphocytes have not gone through all the stages of immunogenesis, and, consequently, differentiation, which is typical for periods of high immunity tension (Fig. 1).

The established patterns of the dynamics of the luminescence intensity of blood cells, fluorochromated by AO, can manifest themselves in several cell generations and other types of the cell population, for example, in highly renewable epithelial tissue. Therefore, this luminescent method is very informative for studying cell biology under the influence of endogenous and exogenous environmental factors.

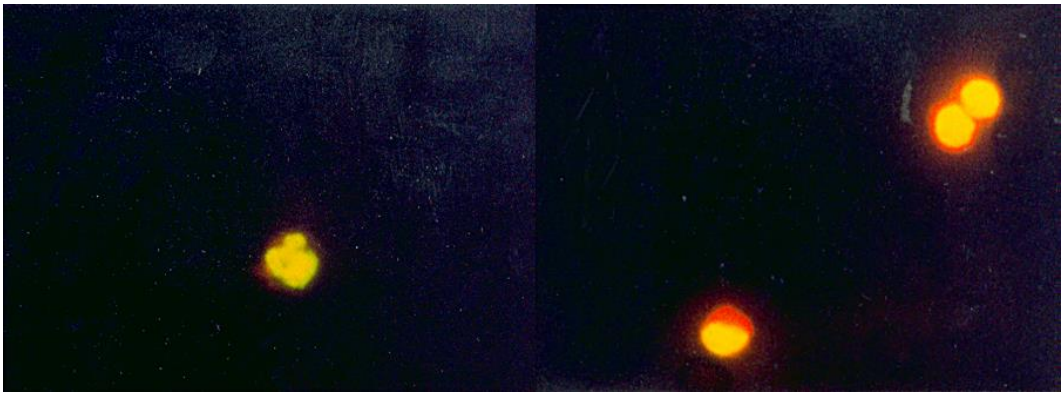

Fig. 1. On the left – an activated neutrophil, on the right – activated lymphocytes

Conclusions

1. Variations in the level of I = 640 nm of the blood leukocytes of the operated patients determined the value of the A-parameter with the relative stability of I = 530 nm; therefore, the activity of cellular immune responses can be determined by the accumulation of single-stranded nucleic acids.
2. The dynamics of changes of I = 640 nm in segmented neutrophils repeated that in lymphocytes but was significantly less in amplitude and duration of the reaction, which is associated with alterations in their differentiated state and participation in immune reactions.
3. Significant radical changes of I = 640 nm in blood lymphocytes, which occurred during the traumatic period of the operation, persisted for a week after the operation, indicating the degree of participation of the immune system in reparative regeneration.

Conflict of interest

The authors declare that they have no conflict of interest.

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