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Interconnection of the immune system and the intensity of the oxidative processes under conditions of prolonged exposure to small doses of radiation

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This research studied the interrelationship between the immune and oxidative-antioxidant systems in a group of individuals who had lived for a long time in areas contaminated with radionuclides after the Chernobyl catastrophe and as a result experienced prolonged exposure to small doses of ionizing radiation. We have examined a group of 100 students aged 18–24, where 50 of them formed the control group and the remaining 50 belonged to the experimental group as they arrived from the territories of enhanced radioecological control (IV radiation zone, density of soil contamination by isotope ^{137}Cs $3.7 \times 10^4 - 18.5 \times 10^4$ Bq/m^2). Here we determined the level of cortisol, leukocytes and their populations, the levels of lymphocyte subpopulations with phenotypes CD3+, CD5+, CD4+, CD8+, CD16+, CD72+, immunoregulatory index CD4+/CD8+, indicators of phagocytic activity of neutrophils and monocytes, IgG (H), IgM (H), IgA (H), malondialdehyde (MDA), ceruloplasmin (CP), transferrin (Tr), sulfhydryl (SH); and also calculated the oxidative stress index (OSI). We performed the analysis twice: in the absence/presence of additional emotional stress such as an examination session. The studies showed an increase in the oxidative stress index in the group examined from the experimental cluster, especially in terms of emotional stress. At the same time, the neutrophil level increased, but phagocytic activity of neutrophils and monocytes, the relative and absolute number of lymphocytes with phenotypes CD3+, CD5+, CD4+, CD4+/CD8+, and IgG levels decreased. Consequently we revealed the negative correlation between the indexes of oxidative stress in the group of examined (the oxidative stress index (ISO)/the level of malonic dialdehyde (MDA)) and the parameters of phagocytic activity of monocytes, the immunoregulatory index CD4+/CD8+, and the number of lymphocytes with the CD16+ phenotype. In this study we demonstrated the decrease in the participation of ceruloplasmin (CP) as an important antioxidant factor in maintaining the immune homeostasis of the group examined from radiation-contaminated areas compared with control group. The evidence of this is the lack of reliable correlation between ceruloplasmin level and immune system parameters. Moreover we found that radiation-induced intensification of oxidative processes in the experimental group grew in conditions of additional stresses of an emotional nature. Besides, it was accompanied by a significant correlation in the level of oxidative stress and phagocytic activity parameters. Reducing phagocytic activity and the CD4+/CD8+ index on the background of oxidative stress increase can be considered as a sign of immune system ageing, while a decrease in the number of lymphocytes with the CD16+ phenotype is a sign of antitumor defense inhibition. Thus, we draw the conclusion that the inhabitants of the territories of strengthened radioecological control, undergoing exposure to small doses of ionizing radiation from birth, show a significant imbalance of redox homeostasis, which creates the preconditions for immunoreactivity pathology development at the level of both innate and acquired immunity.

Keywords: Chernobyl accident; malonic dialdehyde; ceruloplasmin; T-lymphocytes; phagocytic activity; emotional stress

Introduction

The question of the medical status of the population living on the territories contaminated by radionuclides as the result of the Chernobyl catastrophe is quite controversial. At present, separate settlements of the IV radiation zone are deprived of the status of being affected by the Chernobyl catastrophe. However, some definite changes in the bodies of the local population caused by chronic irradiation in low doses (due to the soil activity of long-lived ^{137}Cs isotope, inhaled radionuclides and radionuclide intakes in food) may be manifested through a remote period of time in the form of more or less pronounced pathologies or pre-pathological conditions (Eheman et al., 2003; Davis et al., 2004). In particular, this concerns the state of the immune system, where significant radiation-induced structural and metabolic dysfunctions in immune-competent cells are probable. Taking into account the integrative activity of the immune system as a factor in maintaining the antigenic homeostasis of the body, any disturbances in one of its components can be reflected in the work of the system as a whole (Manuck et al., 1991; Sheikh Sajjadieh et al., 2009; Balogh et al., 2013).

The results of assessing the effects of major radiation catastrophes really indicate the possibility of immediate and long-term effects on the immune status of the affected people (Kusunoki & Hayashi, 2008; Kusunoki et al., 2010). At the level of nonspecific protection factors, adaptive or pathological changes induced by ionizing radiation are possible (Wang et al., 2013).

It should be taken into consideration that ionizing radiation is considered to be a very powerful stress factor, which potentially can lead to a decrease in immunological surveillance (Duffner, 2004), and

also to cause activation of oxidative processes (Nylund et al., 2014). With additional exogenous effects of a stress character, the risk of immune system impairment increases (Gleeson, 2007). In particular, this relates to the psycho-emotional load, which is considered to be one of the most common modern immune suppressants and stimulating agents to trigger adaptive reactions in the body (Segerstrom & Miller, 2004; Viru & Viru, 2004; Filaire et al., 2009). In parallel, the risk of further activation of oxidative processes increases. Indeed, chronic neural-psychological stress, interacting with adverse environmental factors, can lead to the formation of metabolic syndrome (Beckham et al., 2003). It is known that oxidative stress is involved in the development of metabolic syndrome (Roberts & Sindhu, 2009; Furukawa et al., 2017).

The oxidative processes in the body are normal physiological and biochemical phenomena. In particular, they include the process of lipid peroxidation. In this case, antioxidant systems provide realization of these reactions within the limits of the homeostatic norm. Under conditions of ionizing radiation influence, the development of oxidative processes qualitatively different from spontaneous cells, is probable. Radiolysis products with high oxidation or reduction activity can initiate them. Radiation-induced oxidative processes are particularly clearly manifested in cases of acute irradiation (Combs & Combs, 1986). In the range of small doses, the consequences are rather ambiguous and, obviously, depend on certain additional factors (Stone & Dratz, 1982). Definitely, ionizing radiation, even in small doses, leads to the activation of lipid peroxidation reactions, while the surplus of their products can have a damaging effect, on the background of exhaustion of enzyme and non-enzyme antioxidant systems (Dostert et al., 1991). In addition, among people affected by radiation catastrophes, a significant decrease in the level of haptoglobin in plasma as an important antioxidant protection factor is observed (Nylund et al., 2014).

There is much evidence of the interaction between the immune system and oxidative stress in terms of diseases of different etiologies. Active forms of oxygen are involved in early T-cell activation and proliferation processes modulation. During chronic oxidative stress, neoantigens that stimulate autoimmune reactions are formed (Stewart et al., 2004; Laddha et al., 2013). Acute oxidative stress can suppress immunity and create preconditions for the development of oncological diseases (Cooke et al., 2003; Reiche et al., 2005). Free radicals are produced by the immune cells themselves to kill infectious factors. However, strong oxidants, in particular, free radicals of oxygen, cause additional stressful effects on natural resistance factors, which results in a decrease in their response to antigens (Roy et al., 1991).

In our previous studies, typical signs of stress reactions among individuals aged 18–24 years old who lived on the territory of enhanced radiation control were marked, in particular, raised level of cortisol (Sokolenko & Sokolenko, 2015), which increased further in conditions of additional psycho-emotional stress (Sokolenko, 2015). As a result, signs of immunosuppression were observed in this group, which were accompanied by an imbalance of thyroid status and lipid metabolism, changes in oxidative and antioxidant activity (Sokolenko & Sokolenko, 2015, 2017a; 2017b; Sokolenko 2016).

The purpose of the work is to find out the specifics of interconnection between the parameters of immune and oxidative-antioxidant systems in a group of inhabitants from the radiation-polluted territories under conditions of additional psycho-emotional stress or its absence.

Materials and methods

We examined 100 persons, including a group of people from non-contaminated areas (control group, 50 persons) and inhabitants of territories of the strengthened radio-ecological control (IV radiation zone, density of soil pollution with isotopes ^{137}Cs $3.7 \times 10^4 – 18.5 \times 10^4$ Bq/m^2, 50 persons). All those examined were students of Cherkasy National University, aged 18–24, at the time of research not having any acute diseases. There was no statistically significant difference between volunteers of different sexes (females were examined in the follicular stage of the menstrual cycle), so they were considered as a single group in the future. The winter examination session played the role of additional stress factor, which predetermined the development of psycho-emotional load. 15 ml of venous blood was taken in the morning, before eating. The first analysis of the blood parameters was made in the inter-session period, the second after the first exam. Medical examinations and blood were performed by qualified medical staff at "Edem" sanatorium at Cherkasy National University and the biochemical laboratory of the City Hospital №1 in Cherkasy.

The research was conducted in compliance with the ethical principles of the European Convention and the Helsinki Declaration, those examined agreed to be tested and that the results should be published. The cortisol content in serum was determined by immune enzyme method using "BIO-RAD" set (the USA).

The total number of white blood cells was calculated in Hemocytometer, absolute and relative number of their populations – based on a blood smear (painting by Romanovskyi-Giemsa).

Expression of surface antigens by peripheral blood lymphocyte was determined using the immune fluorescence method using monoclonal antibodies to surface markers of immune cells LT1 (for evaluation of the expression of pan T cell marker CD5), LT3 (for evaluation of the expression of pan T cell marker CD3), LT4 (for evaluation of the expression of T cell helper activity marker CD4), LT8 (for evaluation of the expression of T cell marker of effector / suppressor activity CD8), LNK16 (for evaluation of the expression of natural killer activity marker CD16) and F(ab)2 -fragments of sheep antibody to mouse IgG labeled with FITC ("Sorbent", Moscow). The level of immune globulins in serum was determined by radial immune diffusion on Manchini using monospecific serums against IgG (H), IgM (H), IgA (H).

The phagocytic index (FI is the average number of microorganisms absorbed by one leukocyte) and phagocytic number (FF – the rate of phagocytic leukocytes, which means the number of leukocytes with phagocytic activity to 100) of neutrophils and monocytes were calculated for their ability to absorb yeast cells (SNL "GRANUM", Kharkiv) with smear painting by Romanovskyi-Giemsa.

We studied the components of oxidative processes and antioxidant system in accordance with the method (Korol & Myhal, 2012). And there determined the content of malondialdehyde (MDA), ceruloplasmin (CP), transferrin (Tr) and sulfhydryl (SH) groups.

The calculation of the oxidative stress index (OSI) was made by formula: OSI = MDAe / MDAc: [(CPe / CPc + Td / Trk + SHd / SHk): 3], where OSI is an index of oxidative stress; MDAe – the content of MDA in the experimental group; MDAc – MDA in the control group (average); CPe - content of CP in the experimental group; CPc – CP in control group (average); Tre – content of Tr in the experimental group; Trc – Tr in the control group (average); SH – the content of SH in the experimental group; SHc – SH in the control group (average); 3 – number of components (Korol & Myhal, 2012).

The data are expressed as mean ± standard error (M ± SE). One-way ANOVA was performed to detect statistical significance. Differences with $P < 0.05$ were considered significant. Correlation analysis was performed using the Pearson correlation coefficient.

Results

It was established that the analyzed parameters in the control group were within the limits of the homeostatic norm. People, who had lived on the territories of enhanced radio-ecological control for a long time and were subjected to prolonged exposure to ionizing radiation in small doses showed a significant increase in relative and absolute number of stab and segmental neutrophils, eosinophils and decrease in the relative and absolute number of lymphocytes, phagocytic number of neutrophils, phagocytic index and phagocytic number of monocytes compared with the control group. Also, in the analyzed group, there was a relative suppression of the T-cell immunity, which manifests itself in a decrease of the relative and absolute number of lymphocytes expressing antigens of CD3, CD5, CD4 and CD16. Decreased immune regulatory index CD4+/CD8+ and increased serum immunoglobulin IgG concentration was marked (Table 1).

The analysis of the oxidative-antioxidant balance of the examined groups showed that the level of malondialdehyde in the experimental group had a tendency to increase; however, the difference with the control group did not have any statistical significance. Alongside this, there is no

significant difference from the control data for ceruloplasmin and transferrin; the content of sulfhydryl compounds was significantly lowered (Table 1).

Under conditions of increased psycho-emotional stress caused by the examination sessions, in the control group there were observed changes that did not lead to overrunning of the homeostatic norm. People undergoing prolonged exposure to small doses of ionizing radiation showed a significant increase in the relative and absolute number of stab and segmental neutrophils; reduction of the relative and absolute number of lymphocytes, lymphocytes with phenotypes CD3+, CD5+, CD4+, CD16+, immune regulatory index CD4+/CD8+, serum IgG, phagocytic index and phagocyte number of neutrophils and monocytes. There was also a tendency to increase in the level of malondialdehyde in the control group and a significant increase of the parameter in the group of students who came to study from the territories of enhanced radio-ecological control. That is, peroxidation in this group was more intensive. In both groups, it was compensated by a significant increase in the content of sulfhydryl compounds in serum, however, in the experimental group, the index remained significantly lower than in the control before and after the psycho-emotional load (Table 1).

In the examined students from both groups there were no signify-cant changes in ceruloplasmin. As a result, in the group of people who suffered chronic exposure to small doses of ionizing radiation, the index of oxidative stress increased significantly in comparison with the control group both before stress and after psycho-emotional load.

Table 1
Coefficient of cortisol, immune and oxidative-antioxidant systems of the examined students

Components	Control, n = 50, M ± SE		Experimental group: people undergoing prolonged influence of small radiation doses, n = 50, M ± SE	
	before emotional stress	during emotional stress	before emotional stress	during emotional stress
Cortisol, nmol /l	351.12 ± 10.136	801.25 ± 12.541 *	633.48 ± 22.156 *	884.97 ± 14.884 *,**
Leucocytes, x 10^9 /l	6.60 ± 0.067	6.61 ± 0.055	6.91 ± 0.065 *	7.07 ± 0.166 *
Lymphocytes, %	26.52 ± 0.278	26.15 ± 0.285	23.52 ± 0.275 *	20.36 ± 0.397 *,**
Lymphocytes, x 10^9 /l	1.80 ± 0.036	1.73 ± 0.035	1.66 ± 0.041 *	1.26 ± 0.046 *,**
Monocytes, %	5.71 ± 0.296	5.24 ± 0.310	6.12 ± 0.244	5.56 ± 0.239
Monocytes, x 10^9 /l	0.41 ± 0.035	0.40 ± 0.041	0.43 ± 0.019	0.40 ± 0.045
Stab neutrophils, %	3.55 ± 0.275	4.02 ± 0.302	4.59 ± 0.061*	7.15 ± 0.163 *,**
Stab neutrophils, x 10^9 /l	0.25 ± 0.013	0.29 ± 0,024	0.31 ± 0,019*	0.41 ± 0.029 *,**
Segmental neutrophils, %	62.85 ± 0.496	63.97 ± 0.587	65.22 ± 0.481*	67.33 ± 0.496 *,**
Segmental neutrophils, x 10^9 /l	4.18 ± 0.066	4.34 ± 0.089	4.47 ± 0.052 *	6.03 ± 0.096 *,**
Basophils, %	0.12 ± 0.071	0.15 ± 0.079	0.34 ± 0.101	0.41 ± 0.105
Basophils, x 10^9 /l	0.01 ± 0.005	0.01 ± 0.007	0.02 ± 0.009	0.02 ± 0.009
Eosinophils, %	1.12 ± 0.298	1.23 ± 0.310	2.31 ± 0.206 *	2.86 ± 0.225 *
Eosinophils, x 10^9 /l	0.07 ± 0.018	0.09 ± 0.021	0.12 ± 0.012 *	0.14 ± 0.021
Phagocytic index of neutrophils	5.71 ± 0.305	5.75 ± 0.387	4.70 ± 0.301*	3.77 ± 0.294 *,**
Phagocytic number of neutrophils	76.05 ± 0.497	76.92 ± 0.524	74.52 ± 0.723	71.37 ± 0.755 *,**
Phagocytic index of monocytes	5.50 ± 0.417	5.39 ± 0.594	3.45 ± 0.410 *	2.47 ± 0.159 *,**
Phagocytic number of monocytes	75.33 ± 0.498	74.85 ± 0.513	71.36 ± 0.687*	68.75 ± 0.801 *,**
$CD3^+$, %	66.02 ± 0.457	63.31 ± 0.410 *	62.55 ± 0.610 *	54.27 ± 1.156 *,**
$CD3^+$, x 10^9 /l	1.12 ± 0.027	1.06 ± 0.033	1.00 ± 0.028 *	0.72 ± 0.061 *,**
$CD5^+$, %	69.51 ± 0.597	68.99 ± 0.315	65.69 ± 0.594 *	60.36 ± 1.113 *,**
$CD5^+$, x 10^9 /l	1.36 ± 0.033	1.25 ± 0.054	1.13 ± 0.031 *	0.75 ± 0.073 *,**
$CD4^+$, %	40.29 ± 0.406	33.33 ± 0.455 *	33.77 ± 0.597 *	27.74 ± 1.145 *,**
$CD4^+$, x 10^9 /l	0.80 ± 0.018	0.69 ± 0.058	0.60 ± 0.021 *	0.35 ± 0.031 *,**
$CD8^+$, %	27.48 ± 0.406	23.45 ± 0.310 *	26.85 ± 0.394	26.01 ± 0.518 *
$CD8^+$, x 10^9 /l	0.49 ± 0.019	0.44 ± 0.021	0.49 ± 0.018	0.44 ± 0.036
$CD4^+/CD8^+$	1.67 ± 0.031	1.40 ± 0.022 *	1.38 ± 0.031 *	1.13 ± 0.029 *,**
$CD16^+$, %	18.55 ± 1.023	10.61 ± 0.195 *	14.71 ± 1.045 *	10.22 ± 1.096 *,**
$CD16^+$, x 10^9 /l	0.35 ± 0.041	0.19 ± 0.021 *	0.25 ± 0.017 *	0.10 ± 0.018 *,**
$CD72^+$, %	9.88 ± 0.178	9.63 ± 0.199	10.33 ± 0.365	9.64 ± 1.012
$CD72^+$, x 10^9 /l	0.17 ± 0.015	0.16 ± 0.014	0.19 ± 0.021	0.15 ± 0.026
IgG, mg/ml	10.15 ± 0.198	8.72 ± 0.136 *	11.37 ± 0.464 *	6.49 ± 0.991 *,**
IgM, mg/ml	1.66 ± 0.122	1.47 ± 0.041	1.89 ± 0.145	1.81 ± 0.154 *
IgA, mg/ml	1.81 ± 0.096	1.77 ± 0.055	1.70 ± 0.096	1.64 ± 0.099
Malondialdehyde (MDA), mmol /l	125.41 ± 27.510	150.75 ± 36.410	136.31 ± 14.145	200.65 ± 24.142 **
Ceruloplasmin (CP), g /l	0.24 ± 0.018	0.27 ± 0.029	0.22 ± 0.026	0.21 ± 0.022
Transferrin (Tr) cond. un.	5.33 ± 1.012	5.50 ± 1.018	4.39 ± 0.896	4.25 ± 1.151
SH-groups, mmol /l	2.52 ± 0.027	2.73 ± 0.068*	1.70 ± 0.042 *	2.00 ± 0.061 *,**
Oxidative stress index (OSI), un.	1.03 ± 0.041	1.10 ± 0.053	1.41 ± 0.048 *	1.95 ± 0.059 *,**

Notes: * – $P < 0.05$ compared to control; ** – $P < 0.05$ compared to the coefficient before psycho-emotional load.

Correlation analysis in the inter-session period, under the conditions of absence of additional psycho-emotional load (the graphs show the most informative correlations), showed that there was a significant positive correlation between the level of malonic dialdehyde and the relative and the absolute number of neutrophils. A similar correlation with the level of neutrophils was noted for the index of oxidative stress, and the value of the correlation coefficient is slightly higher. However, the correlation of malonic dialdehyde index and index of oxidative stress with phagocytic activity of neutrophils (phagocytic index and phagocytic number) is negative and doesn't have any statistical significance (Fig. 1).

A slightly different situation is typical for correlations of another population of professional phagocytes of peripheral blood – monocytes. Their correlation (both relative and absolute) with the level of malonic dialdehyde and the index of oxidative stress was negative, and the reliability of the correlation coefficient was noted for the phagocytic index and phagocytic number of monocytes (Fig. 2). The analysis of correlations between indices of oxidation-antioxidant system of the persons examined and lymphocyte subpopulations of peripheral blood showed the following: the malonic dialdehyde and oxidative stress index negatively correlated with the relative and absolute number of lymphocytes with phenotypes CD3+, CD4+, CD8+, CD16+, and immunoregulatory index CD4+/CD8+. Correlation with the level of T lymphocytes with CD8+ phenotype was not statistically significant. In all cases, the negative correlation with the indices of oxidative stress was higher than with malonic dialdehyde level. The highest value of the correlation coefficient was noted for the immune regulatory index CD4+/CD8+ (Fig. 3, 4).

The analysis of correlations, conducted under the conditions of additional psycho-emotional load (during the examination session), showed an increase of the interdependence between the analyzed indices, which was manifested by the increase in the values of the correlation coefficient

(Fig. 5–8). Negative correlations between the indices of oxidative stress and the indices of phagocytic activity of neutrophils (phagocytic index and phagocytic number) were statistically significant (Fig. 5).

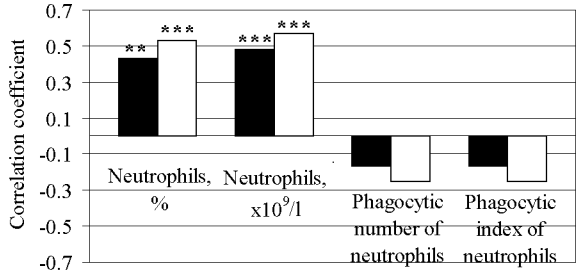

Fig. 1. Correlation between the level of neutrophils, their phagocytic activity and the level of malondialdehyde (black) / oxidative stress index (white) in the group of people who suffered prolonged exposure to small doses of ionizing radiation, to emotional stress (n = 50):
** – reliability of the correlation coefficient, P < 0.01;
*** – reliability of the correlation coefficient, P < 0.001

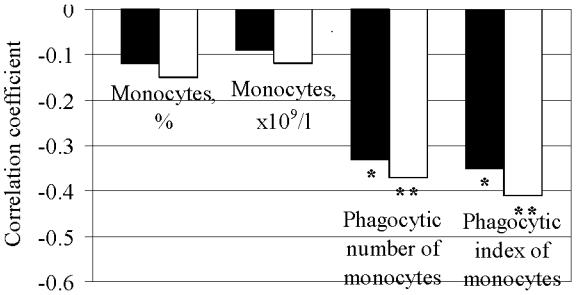

Fig. 2. Correlation between the level of monocytes, their phagocytic activity and the level of malondialdehyde (black) / oxidative stress index (white) in the group of people who suffered prolonged exposure to small doses of ionizing radiation, to emotional stress (n = 50):
* – reliability of the correlation coefficient, P < 0.05;
** – reliability of the correlation coefficient, P < 0.01

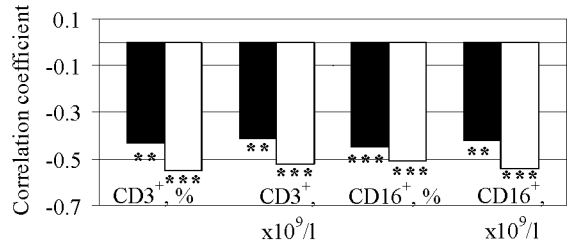

Fig. 3. Correlation between the level of lymphocytes with phenotypes CD3+, CD16+ and the level of malondialdehyde (black) / oxidative stress index (white) in the group of people who suffered prolonged exposure to small doses of ionizing radiation, to emotional stress (n = 50): ** – reliability of the correlation coefficient, P < 0.01;
*** – reliability of the correlation coefficient, P < 0.001

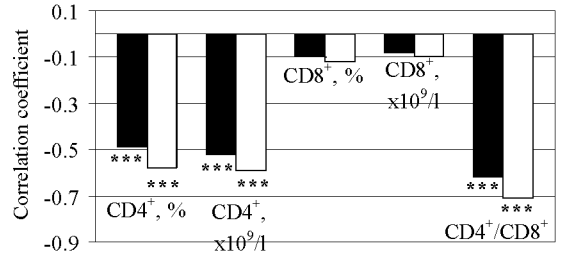

Fig. 4. Correlation between the level of lymphocytes with phenotypes CD4+, CD8+, the immunoregulatory index CD4+/CD8+ and level of malonic dialdehyde (black) / oxidative stress index (white) in the group of people who suffered prolonged exposure to small doses of ionizing radiation, to emotional stress (n = 50):
*** – the reliability of the correlation coefficient, P < 0.001

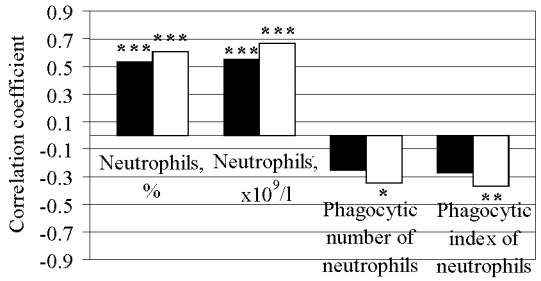

Fig. 5. Correlation between the level of neutrophils, their phagocytic activity and the level of malonic dialdehyde (black) / oxidative stress index (white) in the group of people who suffered prolonged exposure to small doses of ionizing radiation during emotional stress (n = 50): * – reliability of the correlation coefficient, P < 0.05;
** – reliability of the correlation coefficient, P < 0.01;
*** – the reliability of the correlation coefficient, P < 0.001

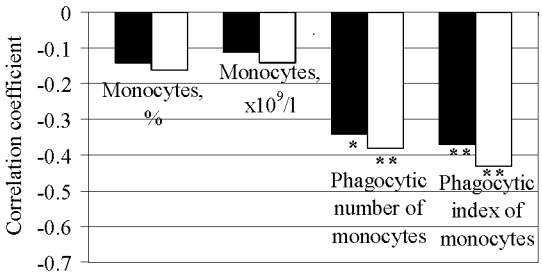

Fig. 6. Correlation between the level of monocytes, their phagocytic activity and the level of malonic dialdehyde (black) / oxidative stress index (white) in the group of people who suffered prolonged exposure to small doses of ionizing radiation during emotional stress:
* – reliability of the correlation coefficient, P < 0.05;
** – reliability of the correlation coefficient, P < 0.01

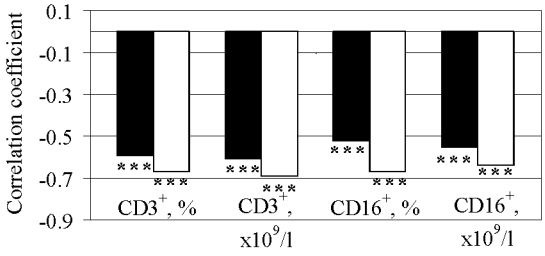

Fig. 7. Correlation between the level of lymphocytes with phenotypes CD3+, CD16+ and the level of malonic dialdehyde (black) / oxidative stress index (white) in the group of people who suffered prolonged exposure to small doses of ionizing radiation during emotional stress;
*** – the reliability of the correlation coefficient, P < 0.001

The analysis of the correlation between the indices of the oxidative-antioxidant system and the level of serum immune globulins showed that during the inter-session period, the values of the coefficient were positive, however, they had no statistical significance (Fig. 9). Under conditions of additional psycho-emotional loading, the value of the correlation coefficient between the level of malonic dialdehyde, the oxidative stress index and the levels of serum immune globulins IgG and IgM changed the sign to negative; regarding IgG, the index has become statistically significant.

The statistical significance of the correlation between the level of ceruloplasmin as an important antioxidant factor and the analyzed immune system parameters was noted only for the control group during the period of intensified psycho-emotional stress (as to immune regulatory index CD4+/CD8+, the phagocytic index of neutrophils, relative and absolute number of lymphocytes with the CD16+) (Fig. 10, 11).

Discussion

Persons who had lived for a long time in the areas contaminated by radionuclides experienced the signs of a chronic stress state. In particu-

lar, we observed an increased level of cortisol and oxidative stress index. The increase in the oxidative stress index was, in turn, provoked by a tendency to increase of malondialdehyde (MDA) on the background of the absence of significant changes in the level of transferrin, ceruloplasmin, as well as a significant reduction of an important protector of oxide process – sulfhydryl compounds. Under the conditions of intensified psycho-emotional load, the intensification of stressful activity was marked. This is evidenced by further increase in the level of stress markers – cortisol and MDA (Table 1).

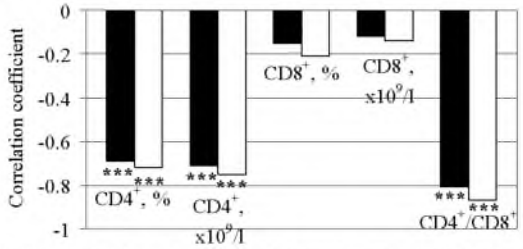

Fig. 8. Correlation between the level of lymphocytes with phenotypes CD4+, CD8+, the immunoregulatory index CD4+/CD8+ and the level of malonic dialdehyde (black) / oxidative stress index (white) in the group of people who suffered prolonged exposure to small doses of ionizing radiation during emotional stress (n = 50):
*** – the reliability of the correlation coefficient, $P < 0.001$

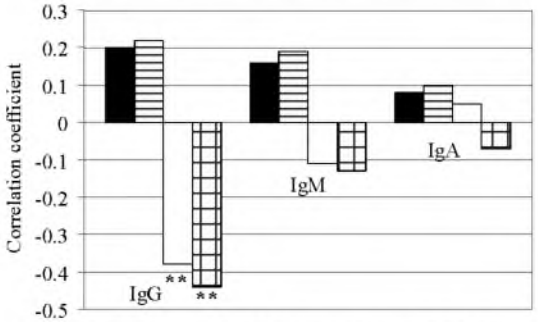

Fig. 9. Correlation between the level of serum immunoglobulins and the level of malodialdehyde (black – before emotional stress, white – during emotional stress) / oxidative stress index (striped – before emotional stress, checked – during emotional stress) in the group of people who suffered prolonged exposure to small doses of ionizing radiation (n = 50); ** – the reliability of the correlation coefficient, $P < 0.01$

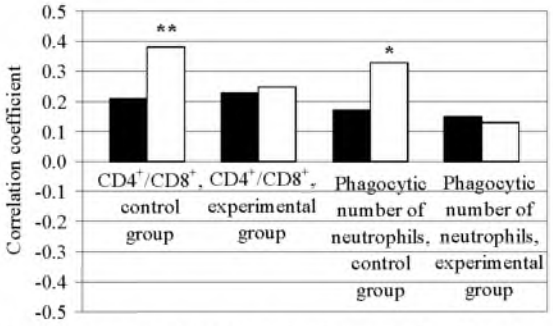

Fig. 10. Correlation between the level of ceruloplasmin and the immunoregulatory index CD4+/CD8+ (black – before emotional stress, white – during emotional stress), and also between ceruloplasmin and phagocytic number of neutrophils (black – before emotional stress, white – during emotional stress) in the control group (n = 50) and in the group of people who suffered prolonged exposure to small doses of ionizing radiation (n = 50); * – reliability of the correlation coefficient, $P < 0.05$; ** – reliability of the correlation coefficient, $P < 0.01$

Increase in corticosteroids level is considered to be one of the most common signs of stress, contributing to the adaptation of the body to environmental changes and reflecting the response to various exogenous stimuli (Filaire et al., 2009; Viru & Viru, 2004). Glucocorticoids are the key factors in stress-induced changes in immunoreactivity. In general, despite the differences in physical and psychological stress factors, their biochemistry and the effects on the body are similar. Prolonged chronic stress results in the same effects that are typical for acute stress conditions. The indicated patterns apply, in particular, to immune system reactions to stress factors (Butcher & Lord, 2004).

Today, the concept of "stress" is defined as the process of modified biochemical homeostasis under the influence of psychological, physiological or environmental stress factors. Any factor potentially dangerous to the body can be classified as a stressor and may activate functioning of homeostatic systems in the body (Rahal et al., 2014).

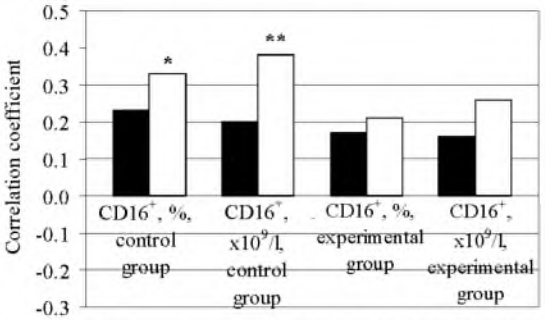

Fig. 11. Correlation between the level of ceruloplasmin and the level of lymphocytes with the CD16+ phenotype (relative and absolute number) in the control group (n = 50) and in the group of people who suffered prolonged exposure to small doses of ionizing radiation (n = 50), before (black) and during (white) emotional stress;
* – reliability of the correlation coefficient, $P < 0.05$;
** – reliability of the correlation coefficient, $P < 0.01$

Definitely, ionizing radiation is among the recognized stressors (Duffner, 2004), and it stimulates the development of oxidative processes and, as a result, leads to the formation of oxidative stress (Stone & Dratz, 1982; Combs & Combs, 1986; Nylund et al., 2014). However, the activation of free radical processes (oxidative stress) is also a typical consequence of the influence of stress factors of another nature (Rahal et al., 2014). In particular, there are findings of immunity inhibition and oxidative DNA damage due to psychological stress (Cooke et al., 2003; Reiche et al., 2005).

Oxidative stress is associated with the etiopathogenesis of many chronic diseases and plays an important role in the aging process (Ceconi et al., 2003). One of the most common targets for oxidative stress are lipids. Their oxidation leads to the formation of a number of secondary metabolites able to exacerbate oxidative damage to biomolecules (Uchida, 2000). Malonic dialdehyde (MDA) is the main well-studied product of the peroxidation of polyunsaturated fatty acids. It is not only a marker of oxidative processes. The interaction of MDA with DNA and proteins is potentially mutagenic and atherogenic (Del Rio & Pellegrini, 2005). We indicated that liquidators of the Chernobyl catastrophe consequences had an increased level of malonic dialdehyde, which created the risk of activation and of inflammatory processes and making them chronic (Emerit et al., 1995). In addition, for this group, an increase of ceruloplasmin and decrease of transferrin in serum was typical (Zueva et al., 2001).

Ceruloplasmin is the main carrier of copper in human plasma. This protein participates in the reaction of the acute phase to stress, and it is believed that some of its physiological functions remain questionable. The antioxidant activity of ceruloplasmin is described (Wood et al., 2006), but some findings suggest this protein may also have pro-oxidant effect and cause oxidative modification of low density lipoprotein (LDL) (Fox et al., 1995). In physiological conditions, ceruloplasmin is an important factor in controlling membrane oxidation of lipids, preventing their peroxidation (Taysi et al., 2002). A significant increase in ceruloplasmin level may indicate an abnormally high oxidative stress (Cunningham et al., 1995). Normally, the level of ceruloplasmin correlates with the level of MDA and transferrin. Cerulloplasmin and transferrin are believed to be important antioxidants that act in interconsistency (Memişoğulları & Bakan, 2004; Somogyi et al., 2007). Important antioxidant components are sulfhydryl groups SH (Birben et al., 2012).

Under the influence of stress factors, all components of the immune response are included in the development of the adaptive response

(Freestone et al., 2008; Bailey, 2016). Indeed, the examined persons from the experimental group showed differences from the control group in parameters of both non-specific and specific immunity.

A significant growth of absolute and relative number of stab and segmental neutrophils on the background of a decrease in the relative and absolute number of lymphocytes is another clear sign of a stressful reaction of moderate intensity. Cortisol induces production of neutrophils in the bone marrow and inhibits their apoptosis in the peripheral blood (Butcher & Lord, 2004). At the same time, neutrophils are one of the important factors in stimulating oxidative stress, which includes myeloperoxidase – their lysosomal enzyme (Lutai et al., 2016). The formation of phagocytes with signs of raised oxidative reactivity may be evidenced by changes in lipid metabolism (Peluso et al., 2012). A positive relationship between the level of lipid peroxidation and the concentration of cholesterol in plasma is reported. Oxidative damage correlates with the level of cholesterol of low density lipoprotein (Hs-LDL) and triglycerides (Fernández-Sánchez et al., 2011). Indeed, we have already shown that the level of total cholesterol and Hc-LDL was increased in the experimental group, and so, the level of Hc-LDL positively correlated with the number of stab neurotrophils (Sokolenko, 2016; Sokolenko & Sokolenko, 2017a). This may indicate stimulated by hypercholesterolemia activation in the development of neutrophils in the bone marrow and spleen, which, in turn, creates preconditions for the development of inflammatory reactions (Tall & Yvan-Charvet, 2015). Proinflammatory factors produced by monocytes and macrophages are involved in the development of oxidative stress that accompanies the process of obesity (Bondia-Pons et al., 2012).

The involvement of neutrophils to the development of oxidative stress in the examined group was confirmed by a positive correlation between the level of these leukocytes with the level of malonic dialdehyde and the oxidative stress index, the value of the correlation coefficient increased in conditions of additional psycho-emotional stress (Fig.1, 5).

The increase in the level of neutrophils in the experimental group was accompanied by a decrease in their phagocyte number and phagocytic index (Table 1). Indicators of phagocytic activity negatively correlated with the level of malonic dialdehyde and the oxidative stress index, in terms of psychoemotional load, the correlation with the index of oxidative stress acquired statistical significance (Fig. 1, 5). Statistically significant correlation of malonic dialdehyde and oxidative stress index with indicators of phagocytic activity of another population of professional phagocytes – monocytes (both in conditions of additional psycho-emotional load presence/absence) was negative (Fig. 2, 6).

The literature gives contradictory data on the sensitivity of the phagocytic component of the natural resistance of the body to the effects of ionizing radiation, especially of low intensity (Liu et al., 2013; Wang et al., 2013; Wunderlich et al., 2015; Pinto et al., 2016). In chronic low-dose irradiation, neutrophil level often increases on the background of weakening of monocytic and lymphatic reactions (Jahns et al., 2011; Heylmann et al., 2014). However, radiation-induced stimulation of proliferative activity of granulocytic hemoptysis is accompanied by a significant number of errors in mitosis (Ghosh & Pyasi, 2016). One of the reasons may be radiation induced free radical processes.On the one hand, free radicals, formed as a result of oxidative processes, are involved in the process of neutrophils and macrophages phagocytosis On the other hand, oxidative stress, acting on certain nuclear factors of phagocytes, may negatively affect their activity. The autocatalytic free radical process, which is marked by MDA level, is peroxide oxidation of lipids, which damages the membranes of immune cells and leads to changes in their metabolic activity, the ability to recognize antigens, etc. (Knight, 2000).

Oxidative stress related to the etiopathogenesis of many chronic diseases plays an important role in the aging process (Ceconi et al., 2003) and has recently been considered to be its biomarker (Pandey & Rizvi, 2010). On the other hand, in the group of elderly people we note suppressing of phagocytosis by cortisol, which leads to inhibiting the production of active forms of oxygen by neutrophils. To the signs of ageing we also refer a decrease in the number of Toll-like receptors on macrophages, and, accordingly, a decrease in their phagocytic activity (Butcher & Lord, 2004). Thus, stress-induced inhibition of phagocytic activity of neutrophils and monocytes on the background of raised cortisol level in the group of students examined from the territory of enhanced radio-ecological control can be characterized as a sign of immune system ageing.

To estimate the potential acceleration of the ageing processes, stimulated by oxidative stress and associated pathological processes (obesity, tumors, diabetes) the antioxidant capacity of blood plasma is marked (Pandey & Rizvi, 2010). One of its important indicators is the level of ceruloplasmin. The antioxidant function of ceruloplasmin in immune processes is caused by the ability to interact with oxygen radicals. In this case, the cells are protecting themselves from excessive peroxidation of lipids with superoxide and other radicals released by neutrophils and macrophages during the inflammation reaction. There are data as to ceruloplasmin participation in direct regulation of oxidative metabolism of phagocytes due to the impact on enzymes associated with their plasma molecule (Saenko et al., 1994). An increase of ceruloplasmin in serum helps to increase the phagocytic activity of neutrophils (Munoz et al., 2007), however, it can also lead to a decrease of their level in peripheral blood flow (Turnlund et al., 2004).

In our case, ceruloplasmin significantly correlated with the phagocytic index of neutrophils only in the control group under conditions of additional emotional stress (Fig. 10). Its level in the experimental group did not differ significantly from the control, and it did not change in conditions of additional emotional load. Taking into account the increased oxidative stress index in this group, this effect may indicate the leveling of this important antioxidant system factor in maintaining proper redox homeostasis and the activity of congenital immunity factors under prolonged influence of small doses of ionizing radiation.

The detected decrease of relative and absolute number of T-lymphocytes with CD3+, CD5+, CD4+ and immune regulatory index CD4+/CD8+ in the experimental group in comparison with the control corresponds with the literature as to selective elimination of the most radiolucent subpopulations of lymphocytes (Frenkel et al., 2005).

Antibody CD3 is a major marker of functionally mature T-lymphocytes and it is absolutely essential for the activation of αβ-TCR T-cell receptor (Dopfer et al., 2014). Antibody CD5 plays an important role in the control of T-lymphocyte autotolerance (Henderson et al., 2015) and also in the regulation of integrated T-cell and B-cell homeostasis due to interaction with antigen CD72 (Zheng et al., 2014). Antigen CD4 is a marker of helper T-lymphocytes – a subpopulation that triggers the formation of the acquired immune response, stimulates B cells to antibody production, triggers and supports the reactions of effector CD8+ -T cells, regulates the macrophage function and, at the same time, interacts with them, provides a comprehensive immune response against a wide range of pathogenic microorganisms, controls its intensity and duration. Lymphocytes with the CD4+ phenotype are important mediators of immunological memory, when their number is reduced or their function is depressed, the individual becomes more susceptible to a wide range of infectious factors (King et al., 2008; Zhu et al., 2010; Yamane & Paul, 2012). T-cells with the CD4+ phenotype have an increased susceptibility to radiation-induced selective elimination (Sheikh Sajjadieh et al., 2009). The revealed negative correlation between the number of T-lymphocytes with the phenotypes of CD3+, CD4+ and the indices of oxidative processes in the experimental group (MDA level and the oxidative stress index) affirms the possibility of participation of lipid peroxidation oxidation activation under conditions of chronic radiation exposure in small doses. The effect is intensified in the conditions of an additional psycho-emotional load, as is shown by the growth in the value of the correlation coefficient (Fig. 3, 4, 7, 8).

According to the literature, the level of MDA and other oxidative factors may affect the proliferative activity of lymphocytes (Lee & Wan, 2000). Oxidative factors produced by phagocytes can induce early expression of genes and proliferation of T-lymphocytes, as well as determine their immune competence. In particular, it concerns helper T-cells (Staite et al., 1987). Excessive free radical and oxidative factors can damage the DNA and the integrity of cell membranes of immune cells (Backer & Weinstein, 1980).

The absence of a reliable correlation with the oxidation parameters of the second major subpopulation of functionally-mature T-lympho-

cytes with the CD8+ phenotype can be explained by their relative radio resistance (Bogdándi et al., 2010). T-lymphocytes with the CD8+ phenotype are able to produce suppressor influence, have an acute cytotoxic activity against infected and tumor cells (Joosten et al., 2007; Kapp & Bucy, 2008; Joosten et al., 2010; Davidsson et al., 2013; Boer et al. 2015). There is information as to the activation of CD8+ T cells in conditions of obesity (Nishimura et al., 2009), and the proatherrogenic changes in the lipid profile that we earlier revealed, could be respecttively involved in the implementation of the revealed effects on T-cell immunity.

The interrelation of lymphocytes with phenotypes CD4+ and CD8+ (immune regulatory index CD4+/CD8+) now is considered to be an important indicator of optimal activity of specific immunity or development of certain diseases (such as cancer), and it is also a marker of susceptibility to exogenous influences (Kidd & Vogt, 1989; Tinago et al., 2014). In our case, the reliable negative correlation of the immunoregulatory index with both the level of malonic dialdehyde and the oxidative stress index is observed (Fig. 4). The value of the correlation coefficient increased with additional emotional stress (Fig. 8). Taking into account the significant positive correlation between the immunoregulatory index and the level of ceruloplasmin in the control group (under conditions of emotional stress), we can talk about the importance of the antioxidant system in maintaining the balance of T-cell immunity. The absence of such a correlation in the experimental group indicates the inhibition of their redox homeostasis (Fig. 10).

Reduction in the CD4+/CD8+ index may be another sign of immune system ageing (Bellingrath et al., 2010; Nakata et al., 2011). As noted above, ageing of the immune system is also characterized by activation of oxidative processes. The ability of the body to maintain proper functional activity and the processes of T-lymphocytes differenttiation largely depends on the ability to counteract the excessive peroxide oxidation of lipids (Wang et al., 2011). Taking into account the overrunning of CD4+/CD8+ immune regulatory index the lower limit of homeostatic norm (in conditions of additional emotional load), in many examined from the territory of enhanced radio-ecological control, we can speak about the preconditions for the development of their immunodeficient states, caused as well by stress-induced activation of oxidative processes.

One of the significantly lowered parameters in the experimental group compared to the control (with subsequent decrease in conditions of additional psycho-emotional loading), is the level of lymphocytes with the phenotype CD16+ (Table). Antigen CD16 is an important marker for natural killers (NK cells) (Romee et al., 2013). Natural killers are characterized by their high cytolytic activity against tumor cells and function on the edge of congenital and acquired immunity (Subleski et al., 2011). In the experimental group, the cells with the CD16+ phenotype showed a significant negative correlation with the level of malonic dialdehyde and the oxidative stress index, besides, the value of the correlation coefficient increased with additional psycho-emotional stress (Fig. 3, 7). In the control group, under the conditions of psycho-emotional stress, a significant positive correlation between the number of lymphocytes with the CD16+ phenotype and the level of ceruloplasmin was noted. For people from the experimental group, the effect was not marked (Fig. 11). According to the literature, natural killers have the highest level of ceruloplasmin expression on the membranes among all lymphocytes. So, these immune system cells have a greater potential for the anti- or prooxidant activity of the body's natural resistance (Banha et al., 2008). Thus, the individuals who have undergone prolonged influence of small doses of ionizing radiation, have inhibited function of lymphocytes with the CD16+ phenotype involvement in maintaining redox homeostasis under additional stressful effects.

The analysis of the humoral immunity revealed a significant correlation with the indices of oxidative processes in the experimental group: a negative correlation between the serum IgG level and the level of malonic dialdehyde and the oxidative stress index, in conditions of additional psycho-emotional stress (Fig. 9). Increased levels of IgG compared to controls, have been observed in the group of students examined from the territories of enhanced radio-ecological control, which was characterized as a compensatory factor of the cellular immunity parameters decrease (Table 1). In addition, according to the literature, the increase in the level of IgG autoantibodies against oxidized low density lipoproteins, on the background of the growth level of malonic dialdehyde is possible (Leonard & Maes, 2012). In conditions of psycho-emotional stress, the level of IgG in the experimental group significantly decreased (Table 1). Taking into account the revealed correlations, one can make assumptions about the participation in this effect the further intensification of oxidative processes.

The growth of correlation interrelationship between the analyzed parameters of the immune system of the students examined from the experimental group and oxidation-antioxidant balance in terms of psychoemotional stress confirms the further intensification of oxidative processes induced by the chronic effects of low doses of radiation. Ionizing radiation is a recognized physical factor for the development of free radical processes and oxidative stress (Stone & Dratz, 1982; Combs & Combs, 1986; Robbins & Zhao, 2004; Nylund et al., 2014). The initiation of response to physiological and physical stress factors is often subconscious and of a purely biological nature (Filaire et al., 2009). However, the increase in the level of anxiety to the verge of depression in 10.0–20.6% (according to different criteria and depending on the age) of Ukrainian schoolchildren aged 7–17 years who are inhabitants of areas contaminated with radionuclides, testifies the presence of additional emotional reactions (Contis & Foley, 2015). Psychological stress factors provide an additional cognitive assessment with the prediction of adverse effects and are also evaluated as a risk factor for the organism, which allows for coordinated behavioural and physiological responses (Filaire et al., 2009).

In depressive states, oxidative stress is often the result of a biological imbalance between active forms of oxygen and antioxidants formed during the activation of immune factors. The inability of cells to adapt to the changes in redox homeostasis and their subsequent death stimulate the inflammation processes. Accordingly, the activation of immune proinflammatory factors and increased oxidative stress act synergistically in the pathogenesis of depression (Leonard & Maes, 2012; Bakunina et al., 2015).

Intensive physical activity can also activate oxidative stress. In this case, there are certain bonds between the oxidizing factors, the antioxidant system and the cellular immune response. In particular, athletes 30 minutes after training check peroxidation, an increase in antioxidant parameters and a decrease in the number of T-lymphocytes with the phenotypes CD3+, CD4+, CD8+, as well as NK cells. Mechanisms underlying the revealed changes are considered multifactorial and related not only to hormonal and metabolic changes due to muscle activity but also to oxidative stress reactions and changes in gene expression (Vider et al., 2001). In our previous research there was observed a moderate decrease in the cellular immunity indices for students from territories contaminated with radionuclides during physical training (Sokolenko & Sokolenko, 2016). However, such changes were effectively restored and, apparently, were induced not by oxidative processes, but by the migration of immune cells.

The involvement of their thyroid status in the revealed characteristics of the interrelationship between the oxidative processes and the immune system of the examined is possible. It is known that the thyroid gland takes part in the development of oxidative stress by stimulating several enzyme systems (De Vito et al., 2011), we also indicate the possibility of the impact of this phenomenon on immune cells proliferation and migration of (Barreiro et al., 2011). In our previous reports, the relationship between the thyroid status of individuals from radiation-contaminated territories, their lipid profiles and the immune system was characterized (Sokolenko & Sokolenko, 2017a, 2017b).

For a detailed analysis of the development of oxidative processes and their influence on the immune system at dosed physical loads of moderate intensity, and also, depending on the thyroid status, additional research is necessary.

In general we can mention that although reactive oxygen types are associated with the pathogenesis of many diseases, their absence in blocking free radicals release from activated phagocytes leads to a violation of the ability of innate immunity factors to eliminate pathogenic microorganisms. Thus, the balance between reactive oxygen

types production and antioxidant protection factors is important (Ferrari et al., 2011). The received data show some definite imbalance of this process in the population of the territories contaminated by radionuclides as a result of the Chernobyl accident.

Conclusions

The prolonged impact of small doses of ionizing radiation due to prolonged residence on the territories contaminated by radionuclides as a result of the Chernobyl accident leads to a stress-induced intensification of oxidative processes, which in turn is reflected on the immune system. Negative correlation of the oxidative stress index and the level of malondialdehyde with the parameters of phagocytic activity of neutrophils and monocytes, the immune regulatory index of CD4+/CD8+ and the number of lymphocytes with the phenotype CD16+ testifies to the participation of oxidative processes in reducing these parameters in individuals from territories with enhanced radio-ecological control. Radiation-induced intensification of oxidative processes grows in conditions of additional stresses of an emotional nature and causes effects that are a sign of ageing of the immune system and antitumor protection inhibition The people undergoing the prolonged influence of small doses of ionizing radiation, show an imbalance of redox homeostasis, which creates preconditions for pathological development of immune reactivity both at the level of innate and acquired immunity.

References

Backer, J. M., & Weinstein, I. B. (1980). Mitochondrial DNA is a major cellular target for a dihydrodiol-epoxide derivative of benzo [a] pyrene. Science, 209(4453), 297–299.

Bailey, M. T. (2016). Psychological stress, immunity, and the effects on indigenous microflora. Advances in Experimental Medicine and Biology, 874, 225–246.

Bakunina, N., Pariante, C. M., & Zunszain, P. A. (2015). Immune mechanisms linked to depression via oxidative stress and neuroprogression. Immunology, 144(3), 365–373.

Balogh, A., Persa, E., Bogdándi, E. N., Benedek, A., Hegyesi, H., Sáfrány, G., & Lumniczky, K. (2013). The effect of ionizing radiation on the homeostasis and functional integrity of murine splenic regulatory T cells. Inflammation Research, 62(2), 201–212.

Banha, J., Marques, L., Oliveira, R., de Fátima Martins, M., Paixão, E., Pereira, D., Malho, R., Penque, D., & Costa, L. (2008). Ceruloplasmin expression by human peripheral blood lymphocytes: A new link between immunity and iron metabolism. Free Radical Biology and Medicine, 44(3), 483–492.

Barreiro Arcos, M. L., Sterle, H. A., Paulazo, M. A., Valli, E., Klecha, A. J., Isse, B., Pellizas, C. G., Farnas, R. N., & Cremaschi, G. A. (2011). Cooperative nongenomic and genomic actions on thyroid hormone mediated modulation of T cell proliferation involve up-regulation of thyroid hormone receptor and inducible nitric oxide synthase expression. Journal of Cellular Physiology, 226(12), 3208–3218.

Beckham, J. C., Taft, C. T., Vrana, S. R., Feldman, M. E., Barefoot, J. C., Moore, S. D., Mozley, S. L., Butterfield, M. I., & Calhoun, P. S. (2003). Ambulatory monitoring and physical health report in Vietnam veterans with and without chronic posttraumatic stress disorder. Journal of Traumatic Stress, 16(4), 329–335.

Bellingrath, S., Rohleder, N., & Kudielka, B. M. (2010). Healthy working school teachers with high effort–reward-imbalance and overcommitment show increased pro-inflammatory immune activity and a dampened innate immune defence. Brain, Behavior, and Immunity, 24(8), 1332–1339.

Birben, E., Sahiner, U. M., Sackesen, C., Erzurum, S., & Kalayci, O. (2012). Oxidative stress and antioxidant defense. World Allergy Organization Journal, 5(1), 9.

Boer, M. C., Joosten, S. A., & Ottenhoff, T. H. (2015). Regulatory T-cells at the interface between human host and pathogens in infectious diseases and vaccination. Frontiers in Immunology, 6, 217.

Bogdándi, E. N., Balogh, A., Felgyinszki, N., Szatmári, T., Persa, E., Hildebrandt, G., Safrany, G., & Lumniczky, K. (2010). Effects of low-dose radiation on the immune system of mice after total-body irradiation. Radiation Research, 174(4), 480–489.

Bondia-Pons, I., Ryan, L., & Martinez, J. A. (2012). Oxidative stress and inflamemation interactions in human obesity. Journal of Physiology and Biochemistry, 68(4), 701–711.

Butcher, S. K., & Lord, J. M. (2004). Stress responses and innate immunity: Aging as a contributory factor. Aging Cell, 3(4), 151–160.

Ceconi, C., Boraso, A., Cargnoni, A., & Ferrari, R. (2003). Oxidative stress in cardiovascular disease: Myth or fact? Archives of Biochemistry and Biophysics, 420(2), 217–221.

Combs Jr, G. F., & Combs, S. B. (1986). The role of selenium in nutrition. Academic Press, Orlando.

Contis, G., & Foley Jr, T. P. (2015). Depression, suicide ideation, and thyroid tumors among Ukrainian adolescents exposed as children to Chernobyl radiation. Journal of Clinical Medicine Research, 7(5), 332.

Cooke, M. S., Evans, M. D., Dizdaroglu, M., & Junec, J. (2003). Oxidative DNA damage: Mechanisms, mutations, and disease. FABEB Journal, 17, 1195–1214.

Cunningham, J., Leffell, M., Mearkle, P., & Harmatz, P. (1995). Elevated plasma ceruloplasmin in insulin-dependent diabetes mellitus: Evidence for increased oxidative stress as a variable complication. Metabolism, 44 (8), 996–999.

Davidsson, S., Ohlson, A. L., Andersson, S. O., Fall, K., Meisner, A., Fiorentino, M., Andren, O., & Rider, J. R. (2013). CD_4 helper T cells, CD_8 cytotoxic T cells, and $FOXP_3^+$ regulatory T cells with respect to lethal prostate cancer. Modern Pathology, 26(3), 448–455.

Davis, S., Stepanenko, V., Rivkind, N., Kopecky, K. J., Voillequé, P., Shakhtarin, V., Parshkov, E., Kulicov, S., Lushnikov, E., Abrosimov, A., Troshin, V., Romanova, G., Doroschenko, V., Proshin, A., & Tsub, A. (2004). Risk of thyroid cancer in the Bryansk Oblast of the Russian Federation after the Chernobyl Power Station accident. Radiation Research, 162(3), 241–248.

De Vito, P., Balducci, V., Leone, S., Percario, Z., Mangino, G., Davis, P. J., Davis, F. B., Affabris, E., Luly, P., Pedersen, J. Z., & Incerpi, S. (2012). Nongenomic effects of thyroid hormones on the immune system cells: new targets, old players. Steroids, 77(10), 988–995.

Del Rio, D., Stewart, A. J., & Pellegrini, N. (2005). A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress. Nutrition, Metabolism and Cardiovascular Diseases, 15(4), 316–328.

Dopfer, E. P., Hartl, F. A., Oberg, H. H., Siegers, G. M., Yousefi, O. S., Kock, S., Fiala, G. J., Garcilan, B., Sandstrom, A., Alarcon, B., Regueiro, J. R., Kabelitz, D., Adams, E. J., Minguet, S., Wesch, D., Fisch, P., & Schamel, W. W. A. (2014). The CD_3 conformational change in the γδ T cell receptor is not triggered by antigens but can be enforced to enhance tumor killing. Cell Reports, 7(5), 1704–1715.

Dostert, P., Benedetti, M. S., & Frigerio, E. (1991). Effect of L-dopa, oxyferriscorbone and ferrous iron on *in vivo* lipid peroxidation. Journal of Neural Transmission/General Section JNT, 84, 119–128.

Duffner, P. K. (2004). Long-term effects of radiation therapy on cognitive and endocrine function in children with leukemia and brain tumors. The Neurologist, 10(6), 293–310.

Eheman, C. R., Garbe, P., & Tuttle, R. M. (2003). Autoimmune thyroid disease associated with environmental thyroidal irradiation. Thyroid, 13(5), 453–464.

Emerit, I., Oganesian, N., Sarkisian, T., Arutyunyan, R., Pogosian, A., Asrian, K., Levy, A., & Cernjavski, L. (1995). Clastogenic factors in the plasma of Chernobyl accident recovery workers: Anticlastogenic effect of *Ginkgo biloba* extract. Radiation Research, 144(2), 198–205.

Fernández-Sánchez, A., Madrigal-Santillán, E., Bautista, M., Esquivel-Soto, J., Morales-González, Á., Esquivel-Chirino, C., Durante-Montiel, I., Sánchez-Rivera, G., Valadez-Vega, C., & Morales-González, J. A. (2011). Inflammation, oxidative stress, and obesity. International Journal of Molecular Sciences, 12(5), 3117–3132.

Ferrari, C. K., Souto, P. C., França, E. L., & Honorio-França, A. C. (2011). Oxidative and nitrosative stress on phagocytes' function: From effective defense to immunity evasion mechanisms. Archivum Immunologiae et Therapiae Experimentalis, 59(6), 441–448.

Filaire, E., Alix, D., Ferrand, C., & Verger, M. (2009). Psychophysiological stress in tennis players during the first single match of a tournament. Psychoneuroendocrinology, 34(1), 150–157.

Fox, P. L., Mukhopadhyay, C., & Ehrenwald, E. (1995). Structure, oxidant activity, and cardiovascular mechanisms of human ceruloplasmin. Life Sciences, 56(21), 1749–1758.

Freestone, P. P., Sandrini, S. M., Haigh, R. D., & Lyte, M. (2008). Microbial endocrinology: How stress influences susceptibility to infection. Trends in Microbiology, 16(2), 55–64.

Frenkel, M., Ben-Arye, E., Baldwin, C. D., & Sierpina, V. (2005). Approach to communicating with patients about the use of nutritional supplements in cancer care. Southern Medical Journal, 98(3), 289–294.

Furukawa, S., Fujita, T., Shimabukuro, M., Iwaki, M., Yamada, Y., Nakajima, Y., Nakayama, O., Makishima, M., Matsuda, M., & Shimomura, I. (2017). Increased oxidative stress in obesity and its impact on metabolic syndrome. The Journal of Clinical Investigation, 114(12), 1752–1761.

Ghosh, B., & Pyasi, K. (2016). Phagocytic activity of neutrophils in chronic obstructive pulmonary disease. Lung India, 33(1), 114.

Gleeson, M. (2007). Immune function in sport and exercise. Journal of Applied Physiology, 103(2), 693–699.

Henderson, J. G., Opejin, A., Jones, A., Gross, C., & Hawiger, D. (2015). CD_5 instructs extrathymic regulatory T cell development in response to self and tolerizing antigens. Immunity, 42(3), 471–483.

Heylmann, D., Rödel, F., Kindler, T., & Kaina, B. (2014). Radiation sensitivity of human and murine peripheral blood lymphocytes, stem and progenitor cells. Biochimica et Biophysica Acta – Reviews on Cancer, 1846(1), 121–129.

Jahns, J., Anderegg, U., Saalbach, A., Rosin, B., Patties, I., Glasow, A., Kamprad, M., Scholz, M., & Hildebrandt, G. (2011). Influence of low dose irradiation on differentiation, maturation and T-cell activation of human dendritic cells. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 709, 32–39.

Joosten, S. A., van Meijgaarden, K. E., Savage, N. D., de Boer, T., Triebel, F., van der Wal, A., de Heer, E., Klein, M. R., Geluk, A., & Ottenhoff, T. H. (2007). Identification of a human CD_8^+ regulatory T cell subset that mediates suppression through the chemokine CC chemokine ligand 4. Proceedings of the National Academy of Sciences, 104(19), 8029–8034.

Joosten, S. A., Van Meijgaarden, K. E., Van Weeren, P. C., Kazi, F., Geluk, A., Savage, N. D., Drijfhout, J. W, Flower, D. R, Hanekom, W. A., Klein, M. R., & Ottenhoff, T. H. (2010). *Mycobacterium tuberculosis* peptides presented by HLA-E molecules are targets for human CD_8^+ T-cells with cytotoxic as well as regulatory activity. PLoS Pathogens, 6(2), e1000782.

Kapp, J. A., & Bucy, R. P. (2008). CD_8^+ suppressor T cells resurrected. Human Immunology, 69(11), 715–720.

Kidd, P. G., & Vogt Jr, R. F. (1989). Report of the workshop on the evaluation of T-cell subsets during HIV infection and AIDS. Clinical Immunology and Immunopathology, 52(1), 3–9.

King, C., Tangye, S. G., & Mackay, C. R. (2008). T follicular helper (TFH) cells in normal and dysregulated immune responses. Annual Review of Immunology, 26, 741–766.

Knight, J. A. (2000). Free radicals, antioxidants, and the immune system. Annals of Clinical and Laboratory Science, 30(2), 145–158.

Korol, L. V., & Myhal, L. A. (2012). Sposib intehralnoi otsinky oksydantno-antyoksydantnoho balansu u syrovatsi krovi [Method of integral evaluation of oxidant-antioxidant balance in blood serum]. Patent No 102192 UA, CIP G01N 33/48 (2006.01). No a201205647. 08.05.2012. Publ. 10.06.2013, Bul. No 11.111-117 (in Ukrainian).

Kusunoki, Y., & Hayashi, T. (2008). Long-lasting alterations of the immune system by ionizing radiation exposure: Implications for disease development among atomic bomb survivors. International Journal of Radiation Biology, 84(1), 1–14.

Kusunoki, Y., Yamaoka, M., Kubo, Y., Hayashi, T., Kasagi, F., Douple, E. B., & Nakachi, K. (2010). T-cell immunosenescence and inflammatory response in atomic bomb survivors. Radiation Research, 174(6b), 870–876.

Laddha, N. C., Dwivedi, M., Mansuri, M. S., Gani, A. R., Ansarullah, M., Ramachandran, A. V., Dalai, S., & Begum, R. (2013). Vitiligo: Interplay between oxidative stress and immune system. Experimental Dermatology, 22(4), 245–250.

Lee, C. Y. J., & Wan, F. (2000). Vitamin E supplementation improves cell-mediated immunity and oxidative stress of Asian men and women. The Journal of Nutrition, 130(12), 2932–2937.

Leonard, B., & Maes, M. (2012). Mechanistic explanations how cell-mediated immune activation, inflammation and oxidative and nitrosative stress pathways and their sequels and concomitants play a role in the pathophysiology of unipolar depression. Neuroscience and Biobehavioral Reviews, 36(2), 764–785.

Liu, C., Zhang, C., Mitchel, R. E., Cui, J., Lin, J., Yang, Y., Liu, X., & Cai, J. (2013). A critical role of toll-like receptor 4 (TLR4) and its' *in vivo* ligands in basal radio-resistance. Cell Death and Disease, 4(5), e649.

Lutai, Y. M., Parkhomeko, O. M., Ryzhkova, N. O., Havrylenko, T. I., Irkin, O. I., Kozhukhov, S. M., Stepura, A. O., & Bilyi, D. O. (2016). Vplyv terapii vnutrishnovennym inhibitorom 5-lipoksyhenazy kvertsetynom na funktsiiu endoteliiu, vyrazhenist systemnoho zapalennia ta prooksydantnoho stresu pry hostromu infarkti miokarda z elevatsiieiu ST [Effects of intravenous 5-lipoxygenase inhibitor quercetin therapy on endothelial function, severity of systemic inflammation and oxidative stress in acute st elevation myocardial infarction]. Medytsyna Nevidkladnykh Staniv, 1, 111–119 (in Ukrainian).

Manuck, S. B., Cohen, S., Rabin, B. S., Muldoon, M. F., & Bachen, E. A. (1991). Individual differences in cellular immune response to stress. Psychological Science, 2(2), 111–115.

Memişoğulları, R., & Bakan, E. (2004). Levels of ceruloplasmin, transferrin, and lipid peroxidation in the serum of patients with Type 2 diabetes mellitus. Journal of Diabetes and its Complications, 18(4), 193–197.

Munoz, C., Rios, E., Olivos, J., Brunser, O., & Olivares, M. (2007). Iron, copper and immunocompetence. British Journal of Nutrition, 98(S1), S24–S28.

Nakata, A., Takahashi, M., & Irie, M. (2011). Effort-reward imbalance, overcommitment, and cellular immune measures among white-collar employees. Biological Psychology, 88(2–3), 270–279.

Nishimura, S., Manabe, I., Nagasaki, M., Eto, K., Yamashita, H., Ohsugi, M., Otsu, M., Hara, K., Ueki, K., Sugiura, S., Yoshimura, K., Kadowaki, T., & Nagai, R. (2009). CD_8^+ effector T cells contribute to macrophage recruitment and adipose tissue inflammation in obesity. Nature Medicine, 15(8), 914–920.

Nylund, R., Lemola, E., Hartwig, S., Lehr, S., Acheva, A., Jahns, J., Hildebrandt, G., & Lindholm, C. (2014). Profiling of low molecular weight proteins in plasma from locally irradiated individuals. Journal of Radiation Research, 55(4), 674–682.

Pandey, K. B., & Rizvi, S. I. (2010). Markers of oxidative stress in erythrocytes and plasma during aging in humans. Oxidative medicine and cellular longevity, 3(1), 2–12.

Peluso, I., Morabito, G., Urban, L., Ioannone, F., & Serafi, M. (2012). Oxidative stress in atherosclerosis development: The central role of LDL and oxidative burst. Endocrine, Metabolic and Immune Disorders-Drug Targets, 12(4), 351–360.

Pinto, A. T., Pinto, M. L., Cardoso, A. P., Monteiro, C., Pinto, M. T., Maia, A. F., Castro, P., Figueira, R., Monteiro, A., Marques, M., Mareel, M., dos Santos, S. G., Seruca, R., Barbosa, M. A., Rocha, S., & Olivera, M. J. (2016). Ionizing radiation modulates human macrophages towards a pro-inflammatory phenotype preserving their pro-invasive and pro-angiogenic capacities. Scientific Reports, 6, 18765.

Rahal, A., Kumar, A., Singh, V., Yadav, B., Tiwari, R., Chakraborty, S., & Dhama, K. (2014). Oxidative stress, prooxidants, and antioxidants: The interplay. BioMed Research International, 2014, e761264.

Reiche, E. M. V., Morimoto, H. K., & Nunes, S. M. V. (2005). Stress and depression-induced immune dysfunction: Implications for the development and progression of cancer. International Review of Psychiatry, 17(6), 515–527.

Robbins, M. E. C., & Zhao, W. (2004). Chronic oxidative stress and radiation-induced late normal tissue injury: A review. International Journal of Radiation Biology, 80(4), 251–259.

Roberts, C. K., & Sindhu, K. K. (2009). Oxidative stress and metabolic syndrome. Life Sciences, 84(21–22), 705–712.

Romee, R., Foley, B., Lenvik, T., Wang, Y., Zhang, B., Ankarlo, D., Luo, X., Cooley, S., Verneris, M., Walcheck, B., & Miller, J. (2013). NK cell CD_{16} surface expression and function is regulated by a disintegrin and metalloprotease-17 (ADAM17). Blood, 121(18), 3599–3608.

Roy, R. M., Petrella, M., & Ross, W. M. (1991). Modification of mitogen-induced proliferation of murine splenic lymphocytes by *in vitro* tocopherol. Immunopharmacology and Immunotoxicology, 13.4, 531–550.

Saenko, E. L., Skorobogat'ko, O. V., Tarasenko, P., Romashko, V., Zhuravetz, L., Zadorozhnaya, L., Senjuk, O. F., & Yaropolov, A. I. (1994). Modulatory Effects of ceruloplasmin on lymphocytes. Neutrophils and monocytes of patients with altered immune status. Immunological Investigations, 23(2), 99–114.

Segerstrom, S. C., & Miller, G. E. (2004). Psychological stress and the human immune system: A meta-analytic study of 30 years of inquiry. Psychological Bulletin, 130(4), 601.

Sheikh Sajjadieh, M. R., Kuznetsova, L. V., Bojenko, V. B., Gydz, N. B., Titkova, L. K., Vasileva, O. U., Uoshenko, I. I., & Drachyk, T. P. (2009). Effect of ionizing radiation on development process of T-cell population lymphocytes in Chernobyl children. Iranian Journal of Radiation Research, 7(3), 127–133.

Sokolenko, V. L. (2016). Pokaznyky kholesterynu ta imunnoyi systemy u osib z oznakamy veheto-sudynnoyi dystoniyi, shcho prozhyvaly na terytoriyakh, zabrudnenykh radionuklidamy [Cholesterol rate and immune system indices in people with symptoms of vegetative-vascular dystonia, who lived in the territories contaminated with radionuclides]. Svit Medytsyny ta Biolohiyi, 2, 86–90 (in Ukrainian).

Sokolenko, V. L., & Sokolenko, S. V. (2016). Vplyv pomirnykh fizychnykh navantazhen' na pokaznyky imunnoi systemy u meshkantsiv radiatsiino zabrudnenykh terytorij [Influence of moderate physical load on parameters of the immune system among residents of contaminated areas]. Visnyk of Dnipropetrovsk University, Biology, Medicine, 7(1), 48–52 (in Ukrainian).

Sokolenko, V. L., & Sokolenko, S. V. (2017a). Vzaiemozviazok lipidnoho obminu ta tyreoidnoho statusu za prolonhovanoho vplyvu malykh doz radiatsii [The interaction between lipid exchange and thyroid status in the conditions of prolonged influence of small doses of radiation]. Regulatory Mechanisms in Biosystems, 8(2), 231–238 (in Ukrainian).

Sokolenko, V. L., & Sokolenko, S. V. (2017b). Vzaiemozviazok pokaznykiv imunnoji systemy ta tyreoidnoho statusu v osib z radiatsiino-abrudnenykh terytoriji za umov psykhoemotsiinoho navantazhennia [Interactions between immune system parameters and thyroid status in people from radioactive contaminated areas by the conditions of emotional stress]. Fiziolohichnyi Zhurnal, 63(3), 32–39 (in Ukrainian).

Sokolenko, V., & Sokolenko, S. (2015). Aktyvnist radionuklidiv i realizatsiia funktsii imunnoi systemy u meshkantsiv radiatsiino zabrudnenykh terytorii [Radionuclide activity and the immune system functioning in residents of radiation contaminated areas]. Visnyk of Dnipropetrovsk University, Biology, Medicine, 6(2), 93–96 (in Ukrainian).

Somogyi, A., Rosta, K., Pusztai, P., Tulassay, Z., & Nagy, G. (2007). Antioxidant measurements. Physiological Measurement, 28(4), R41.

Staite, N. D., Messner, R. P., & Zoschke, D. C. (1987). Inhibition of human T lymphocyte E rosette formation by neutrophils and hydrogen peroxide. Differential sensitivity between helper and suppressor T lymphocytes. The Journal of Immunology, 139(7), 2424–2430.

Stewart, S. F., Vidali, M., Day, C. P., Albano, E., & Jones, D. E. (2004). Oxidative stress as a trigger for cellular immune responses in patients with alcoholic liver disease. Hepatology, 39(1), 197–203.

Stone, W. L., & Dratz, E. A. (1982). Selenium and non-selenium glutathione peroxidase activities in selected ocular and non-ocular rat tissues. Experimental Eye Research, 35(5), 405–412.

Subleski, J. J., Jiang, Q., Weiss, J. M., & Wiltrout, R. H. (2011). The split personality of NKT cells in malignancy, autoimmune and allergic disorders. Immunotherapy, 3(10), 1167–1184.

Tall, A. R., & Yvan-Charvet, L. (2015). Cholesterol, inflammation and innate immunity. Nature Reviews Immunology, 15(2), 104–116.

Taysi, S., Polat, F., Gul, M., Sari, R. A., & Bakan, E. (2002). Lipid peroxidation, some extracellular antioxidants, and antioxidant enzymes in serum of patients with rheumatoid arthritis. Rheumatology International, 21(5), 200–204.

Tinago, W., Coghlan, E., Macken, A., McAndrews, J., Doak, B., Prior-Fuller, C., Lambert, J. S., Sheehan, G. J., & Mallon, P. W. G. (2014). Clinical, immunological and treatment-related factors associated with normalised CD_4^+/CD_8^+ T-cell ratio: Effect of naive and memory T-cell subsets. PloS One, 9(5), e97011.

Turnlund, J. R., Jacob, R. A., Keen, C. L., Strain, J. J., Kelley, D. S., Domek, J. M., Keyes, W. R., Ensunsa, J. L., Lykkesfeldt, J., & Coulter, J. (2004). Long-term high copper intake: Effects on indexes of copper status, antioxidant status, and immune function in young men. The American Journal of Clinical Nutrition, 79(6), 1037–1044.

Uchida, K. (2000). Role of reactive aldehyde in cardiovascular diseases. Free Radical Biology and Medicine, 28(12), 1685–1696.

Vider, J., Lehtmaa, J., Kullisaar, T., Vihalemm, T., Zilmer, K., Kairane, Č., Landor, A., Karu, T., & Zilmer, M. (2001). Acute immune response in respect to exercise-induced oxidative stress. Pathophysiology, 7(4), 263–270.

Viru, A., & Viru, M. (2004). Cortisol-essential adaptation hormone in exercise. International Journal of Sports Medicine, 25(6), 461–464.

Wang, F. P., Li, L., Li, J., Wang, J. Y., Wang, L. Y., & Jiang, W. (2013). High mobility group box-1 promotes the proliferation and migration of hepatic stellate cells via TLR4-dependent signal pathways of PI3K/Akt and JNK. PloS One, 8(5), e64373.

Wang, R., Dillon, C. P., Shi, L. Z., Milasta, S., Carter, R., Finkelstein, D., McCormick, L. L., Fitzgerald, P., Chi, H., Munger, J., & Green, D. R. (2011). The transcription factor Myc controls metabolic reprogramming upon T lymphocyte activation. Immunity, 35(6), 871–882.

Wood, L. G., Gibson, P. G., & Garg, M. L. (2006). A review of the methodology for assessing in vivo antioxidant capacity. Journal of the Science of Food and Agriculture, 86(13), 2057–2066.

Wunderlich, R., Ernst, A., Rödel, F., Fietkau, R., Ott, O., Lauber, K., Frey, B., & Gaipl, U. S. (2015). Low and moderate doses of ionizing radiation up to 2 Gy modulate transmigration and chemotaxis of activated macrophages, provoke an anti-inflammatory cytokine milieu, but do not impact upon viability and phagocytic function. Clinical and Experimental Immunology, 179(1), 50–61.

Yamane, H., & Paul, W. E. (2012). Cytokines of the γ c family control CD_4^+ T cell differentiation and function. Nature Immunology, 13(11), 1037–1044.

Zheng, M., Xing, C., Xiao, H., Ma, N., Wang, X., Han, G., Chen, G., Hou, C., Shen, B., Li, Y., & Wang, R. (2014). Interaction of CD_5 and CD_{72} is involved in regulatory T and B cell homeostasis. Immunological Investigations, 43(7), 705–716.

Zhu, J., Yamane, H., & Paul, W. E. (2010). Differentiation of effector CD_4 T cell populations. Annual Review of Immunology, 28, 445–489.

Zueva, N. A., Iukhimuk, L. N., Gerasimenko, T. I., Kovalenko, A. N., & Efimov, A. S. (2001). Ceruloplasmin, transferrin and insulin blood levels in those with diabetes mellitus type II and in nondiabetics who participated in the clean-up after the Chernobyl accident. Likars'ka Sprava, (3), 14–16.