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SUBPOPULATIONS OF HUMAN PERIPHERAL BLOOD MONOCYTES AND NATURAL KILLER CELLS IN THE LONG-TERM PERIOD OF CHRONIC EXPOSURE

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Introduction. An increased risk of malignancy and cardiovascular diseases is revealed in exposed individuals from different cohorts. Monocyte and natural killer (NK) cells modulate inflammation and carcinogenesis.

Objective. To evaluate absolute and relative cell counts in monocyte and natural killer cell subpopulations in the peripheral blood of individuals exposed to chronic irradiation.

Materials and methods. Thirty-five persons from the Techa River cohort were examined, divided into three subgroups depending on the radiation absorbed dose calculated to the red bone marrow (RBM) (70–249 mGy; 250–699 mGy; 700–1429 mGy, respectively). The mean age of patients was 74.9 years; the mean value of the radiation absorbed dose to RBM was 542.0±65.3 mGy, while that of the radiation absorbed dose to thymus and peripheral lymphoid organs was 99.7±14.4 mGy. The comparison group consisted of 10 persons without a history of anthropogenic irradiation of similar gender and ethnicity, mean age — 71.8 years.

Results. In the second dose subgroup, the proportion of $CD14^-CD16^+$ monocytes was statistically significantly higher (8.47%) than in the comparison group (5.52%, p=0.014), and the absolute $CD14^-CD16^+$ monocytes count (0.040×10^9/l) was also higher than in the third subgroup (0.018×10^9/l, p=0.044) without correlations with radiation and non-radiation factors. No statistically significant differences of other studied parameters between the groups were revealed.

Conclusion. In persons from the second subgroup the relative number of $CD14^-CD16^+$ monocytes was statistically significantly higher than in the comparison group; the absolute $CD14^-CD16^+$ monocytes count was also higher than in the third subgroup without correlations with factors of a radiation and non-radiation nature. The findings are preliminary.

Keywords: chronic radiation exposure; Techa River; subpopulations; peripheral blood monocytes; natural killer cells; long-term period; molecular markers

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СУБПОПУЛЯЦИИ МОНОЦИТОВ И НАТУРАЛЬНЫХ КИЛЛЕРОВ ПЕРИФЕРИЧЕСКОЙ КРОВИ ЧЕЛОВЕКА В ОТДАЛЕННОМ ПЕРИОДЕ ХРОНИЧЕСКОГО ОБЛУЧЕНИЯ

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Введение. Повышенный риск развития злокачественных новообразований и заболеваний сердечно-сосудистой системы установлен для разных когорт облученных людей. При этом известна важная роль моноцитов и натуральных киллеров в модуляции воспаления и канцерогенеза.

Цель. Оценка абсолютного и относительного количества клеток в субпопуляциях моноцитов и натуральных киллеров в периферической крови у лиц, подвергшихся хроническому радиационному воздействию.

Материалы и методы. Обследованы 33 человека из когорты реки Течи, средний возраст пациентов — 74,9 года. Пациенты были разделены на три подгруппы в зависимости от величины поглощенной дозы облучения в красном костном мозге (70–249 мГр, 250–699 МГр, 700–1429 мГр соответственно), средняя поглощенная доза облучения красного костного мозга — 542,1 ± 65,3 мГр, средняя поглощенная доза облучения тимуса и периферических лимфоидных органов — 99,7 ± 14,4 мГр. Группу сравнения составили 10 человек, средний возраст — 71,8 года, без техногенного облучения в анамнезе, сопоставимых по полу и этнической принадлежности.

Результаты. Во второй дозовой подгруппе доля $CD14^-CD16^+$ моноцитов была статистически значимо выше (8,47%), чем в группе сравнения (5,52%, p = 0,014), а абсолютное количество $CD14^-CD16^+$ моноцитов ($0,040\times10^9$/л) — больше, чем в третьей подгруппе ($0,018\times10^9$/л, p = 0,044). Не обнаружено статистически значимых корреляций указанных показателей с факторами радиационной и нерадиационной природы.

Выводы. У людей из второй подгруппы относительное количество $CD14^-CD16^+$ моноцитов было статистически значимо выше, чем в группе сравнения, в то время как абсолютное число $CD14^-CD16^+$ моноцитов — больше, чем в третьей подгруппе, без статистически значимой взаимосвязи с факторами радиационной и нерадиационной природы.

Ключевые слова: хроническое радиационное воздействие; река Теча; субпопуляции; моноциты периферической крови; натуральные киллеры; отдаленные сроки; молекулярные маркеры

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INTRODUCTION

The increased risk of malignant neoplasms (MN) among inhabitants of settlements located along the Techa River affected by ionizing radiation (IR) is a scientifically identified fact confirmed by epidemiological studies [1, 2]. The works of many authors have revealed a correlation between IR dose and risk of hypertension, ischemic heart disease, and cerebrovascular diseases in exposed people in long-term periods following exposure [3]. However, the mechanism of the influence of radiation exposure on the key immune reactions mediating neoplastic processes and/or the development of cardiovascular pathology in irradiated people in long-term periods of exposure to radiation [4] is still insufficiently studied.

A number of authors emphasize that people exposed to technogenic radioactive contamination affecting the Techa River had lower intracellular oxygen-dependent metabolism of monocytes, lower levels of interleukin-4 (IL-4), higher levels of tumor necrosis factor alpha (TNF-α) and interferon gamma (IFN-γ) in serum [5] as compared to unexposed persons. At the same time, TNF-α is weakly correlated with the absorbed dose of red bone marrow irradiation (RBM) [6].

Monocyte–macrophages are of special interest in studying the pathogenesis of the long-term effects of chronic irradiation. The poorly understood role of these cells in the regulation of hematopoiesis and regeneration of RBM represents a promising area of research [7] both under physiological conditions and when RBM are affected by osteotropic radionuclides. Monocyte–macrophages make a significant contribution to the development of both innate and adaptive immune response reactions. As well as participating in the formation of immunological memory, they recognize a wide range of antigenic substances, participate in antigen presentation to T-lymphocytes and regulation of immune responses depending on the type of reaction, its intensity and duration [8].

For many types of oncopathology, the development of inflammatory process caused by active monocytes/macrophages is understood to potentiate the transformation of premalignant tissue into fully malignant tissue. It has been noted earlier that immune cells play a dual role in the pathogenesis of MN. On the one hand, they are able to efficiently and rapidly recognize damaged, transformed and tumor cells in order to neutralize and eliminate them from the body. On the other hand, immunocytes, which typically provide inflammation and removal of genetically foreign agents from the body, contribute to the formation of a pro-tumor microenvironment by producing cytokines and growth factors that stimulate tumor development. In particular, the macrophage migration inhibitory factor (MIF) produced by macrophages suppresses the expression of the *P53* gene, one of the key regulators of cell cycle and apoptosis, which leads to an insufficiently effective response to DNA damage, increased cell life spans, and consequent accumulation of mutations.

Monocyte–macrophages produce a spectrum of growth factors — in particular, vascular endothelial growth factor (VEGF) — which promotes tumor vascularization and metastasis. In turn, tumor signaling molecules provide chemotaxis of monocytes from peripheral blood to the focus of malignant growth and their differentiation into macrophages. The combination of hypoxia and mediators released by tumor cells initiates reprogramming of *de novo* recruited macrophages of the microenvironment into tumor growth promoters — “tumor-associated macrophages” [9]. The presence of macrophages, mast cells, and neutrophils in the tumor microenvironment is usually associated with increased angiogenesis and poor prognosis. However, some clusters of macrophages in the tumor microenvironment may be associated with tumor regression [10].

The cytolytic activity of natural killer (NK) cells is determined by the balance between activating and inhibitory signals and is realized by perforation of the target cell membrane. NK cells can express CD8 α-chain on the membrane but at a lower density than cytotoxic T-cells. Human NK subpopulations expressing αα-homodimer CD8 have greater cytotoxicity than NK cells without a CD8 molecule on the membrane. $CD38^{+}CD8^{+}$ NK-cells have been reported to have high cytolytic activity [11].

Full activation of immunocytes in response to tumor antigens can lead to tumor cell elimination, whereas ineffective immune responses against the background of chronic age-associated inflammation [12] can lead to tumor progression. Chronically activated cells of innate immunity (monocytes/macrophages, natural killer cells, neutrophils

and others) may contribute to the development of MN by suppressing immune reactions, hyperproduction of reactive oxygen species damaging biological membranes and cell DNA, secretion of growth factors and in other ways. Based on the abovementioned, the study of the peculiarities of the quantitative composition of monocyte subpopulations in irradiated people residing along the Techa River, who have now reached the age of realization of oncogenic effects associated with the impact of IR, is a priority direction of research.

In this work, we aim to evaluate absolute and relative cell counts in the subpopulations of monocyte and NK cells in the peripheral blood of persons affected by chronic radiation exposure in the long-term.

MATERIALS AND METHODS

The study was carried out on the basis of the Ural Scientific and Practical Center of Radiation Medicine of FMBA of Russia. Forty-five chronically exposed rural residents permanently residing in areas along the Techa River, which was subject to technogenic contamination during the 1950s due to the activities of the Mayak Production Association, were examined. For each patient, the individualized doses were preliminarily calculated using the Techa River Dosimetry System-2016 (TRDS-2016) [13].

The main group included 33 people, whose absorbed dose calculated on the RBM at the time of examination was 70 mGy or more. The minimum radiation dose calculated on RBM in people from this group was 88.5 mGy, while the maximum dose was 1429 mGy, and the range of doses calculated on thymus and peripheral/secondary lymphoid organs (TSLO) was from 12 to 460 mGy. To permit a detailed study of the dose-effect relationship, the main group was divided into three subgroups depending on the value of the cumulative radiation dose calculated on the RBM: 1st subgroup — 11 people with doses from 70 to 249 mGy; 2nd subgroup — 10 people with doses from 250 to 699 mGy; 3rd subgroup — 12 people with doses from 700 to 1429 mGy.

The comparison group consisted of 10 people with no history of anthropogenic IR exposure, whose absorbed radiation dose was calculated at an RBM less than 70 mGy. The radiation dose calculated on the RBM in the persons included in the comparison group was in the range from 4 to 55 mGy, while the dose on TSLO was from 1 to 20 mGy.

Table 1. Characteristics of the study groups

Parameter, unit of measurement		Comparison group *n* = 10	Main group *n* = 33
RBM dose, mGy		22.5±5.9	542.1±65.3
Dose for TSLO, mGy		8.7±2.4	99.7±14.4
Gender composition, %	male	20.0	27.3
	female	80.0	72.7
Ethnic composition, %	Slavic	50.0	21.2
	Turkic	50.0	78.8

Table prepared by the authors using their own data

Note: The data are presented as average value (*M* ± *SE*)

The mean age of the examined persons in the comparison group was equal to 71.8 ± 1.2 years; in the main group — 74.9 ± 0.6 years. The studied groups of people did not differ statistically significantly in ethnic and gender composition but differed in age ($p = 0.026$). The data on the value of the average radiation dose at RBM, TSLO, gender and ethnic composition in the studied groups of people are presented in Table 1.

Criteria for exclusion of subjects from the study were: signs of acute inflammatory diseases; chronic diseases in exacerbation; renal or hepatic insufficiency; symptoms of acute cerebral circulatory failure or craniocerebral trauma within three months before the study; confirmed oncological and autoimmune diseases; courses of hormone, antibiotic, chemotherapy and (or) radiotherapy; medical procedures involving the use of ionizing radiation within six months before the study.

Human peripheral blood served as a material for immunologic study. Peripheral blood samples (3 mL) were obtained from the ulnar vein in the morning on an empty stomach into a vacuum tube filled with K3-EDTA (tripotassium ethylenediaminetetraacetic acid). The subpopulation composition of monocytes and natural killer cells in peripheral blood was assessed on a LongCyte C3111 flow cytometer (Chenglang Biotechnology, PR China) after preliminary staining with monoclonal antibodies labeled with fluorochromes: CD14-PE, CD16-PerCP, CD45-APC (Elabscience, PRC) — panel for the analysis of monocytes, CD3-FITC, CD56-PE, CD16-PerCP, CD8-PC7, CD45-APC (Elabscience, PRC), CD38-PO (Exbio, Czech Republic), a panel for NK analysis, followed by lysis of erythrocytes with VersaLyse solution (Beckman Coulter Inc., USA) according to the reagent manufacturers' instructions using standardized methods [14]. On the day of blood collection for the study of immunologic parameters in the clinical diagnostic laboratory of Ural Scientific and Practical Center for Radiation Medicine of FMBA of Russia, patients underwent a general blood count with leukoform counting in accordance with the established procedure [15].

Statistical processing of the data was performed in the Statistica 12 software package (demo version). The data were checked for normal distribution using the Kolmogorov–Smirnov test of agreement. The arithmetic mean (M), minimum and maximum values were used to describe normally distributed data. For data that were statistically significantly different from the normal distribution, median, 25th and 75th percentile values were given.

Student's T-test was used to compare parametric data sets; the Mann-Whitney *U*-test was used for nonparametric data. Qualitative data were compared using the χ^2 criterion. Differences were considered statistically significant when the confidence level (p) was less than 0.05. For correlation analysis of abnormally distributed data, the Spearman rank correlation coefficient was calculated with a confidence level of 5%.

RESULTS

When comparing the values of the studied parameters in chronically irradiated people with different dose loads, a statistically significant increase in the absolute monocyte $CD14^{-}CD16^{+}$ count was found in the peripheral blood of

patients from the second subgroup in comparison with those from the third subgroup (p^* = 0.044), and non-exposed patients (p = 0.014) (Table 2).

However, in the process of analyzing the results of quantitative assessment of monocyte subpopulations in the examined persons with different dose loads, no statistically significant differences were found between the median values of the studied parameters in the main group and the comparison group.

The results of quantitative analysis of subpopulations of natural killer cells expressing CD8 and CD38 activation molecules on cell membranes in the examined persons with different absorbed dose of irradiation are presented in Table 3.

Table 2. Results of quantitative analysis of monocyte subpopulations in the examined persons

Indicator, unit of measure	Comparison group *n* = 10	Main group, *n* = 33			
		Subgroups, radiation doses			Median values within the group
		70–249 mGy, *n* = 11	250–699 mGy, *n* = 10	700–1429 mGy, *n* = 12	
Leukocytes, ×10⁹/L	6.14 (5.25–6.60)	5.96 (5.30–6.51)	5.74 (4.70–6.41)	6.23 (5.45–7.05)	5.96 (5.30–6.51)
Monocytes, %	8.2 (7.00–9.90)	8.20 (6.40–11.00)	7.95 (5.00–8.10)	6.00 (3.50–9.15)	8.00 (5.00–9.00)
Monocytes, ×10⁹/L	0.452 (0.364–0.630)	0.467 (0.376–0.679)	0.417 (0.350–0.464)	0.381 (0.185–0.588)	0.420 (0.330–0.567)
$CD14^+CD16^-$ monocytes, %	67.86 (62.45–74.00)	65.58 (54.96–75.66)	69.48 (62.73–73.63)	66.96 (57.50–75.57)	67.68 (58.36–74.80)
$CD14^+CD16^-$ monocytes, ×10⁹/L	0.336 (0.263–0.376)	0.281 (0.218–0.415)	0.305 (0.187–0.314)	0.267 (0.128–0.328)	0.287 (0.187–0.341)
$CD14^+CD16^+$ monocytes, %	4.77 (2.92–6.13)	4.04 (2.85–8.66)	4.25 (2.26–7.30)	3.92 (2.57–5.81)	4.04 (2.48–7.05)
$CD14^+CD16^+$ monocytes, ×10⁹/L	0.024 (0.007–0.031)	0.032 (0.006–0.034)	0.015 (0.004–0.030)	0.095 (0.007–0.019)	0.014 (0.006–0.023)
$CD14^-CD16^+$ monocytes, %	5.52 (3.30–7.50)	6.56 (3.64–8.93)	8.47 (7.98–11.27) p=0.014	6.25 (2.76–9.01)	7.94 (4.67–9.47)
$CD14^-CD16^+$ monocytes, ×10⁹/L	0.018 (0.012–0.034)	0.036 (0.023–0.042)	0.040 (0.033–0.051) p^*=0.044	0.011 (0.007–0.029)	0.033 (0.009–0.042)

Table prepared by the authors using their own data

Notes:
1 — The data are presented as median (25th–75th percentiles).
2 — p — confidence probability of differences relative to the comparison group (Mann–Whitney U test).
3 — p^* p^*=0.044 — confidence probability of differences with respect to the subgroup of people with the highest doses (Mann–Whitney U test).

Table 3. Results of quantitative analysis of NK subpopulations in the examined persons

Parameter, unit of measure	Comparison group, *n* = 10	Main group, *n* = 33			
		Subgroups, radiation doses			Median values in the group
		70–249 mGy, *n* = 11	250–699 mGy, *n* = 10	700–1429 mGy, *n* = 12	
Lymphocytes, %	37.00 (32.40–45.00)	35.00 (30.00–40.90)	39.15 (29.60–46.00)	36.35 (33.55–38.50)	35.70 (30.20–41.00)
Lymphocyte, ×10⁹/L	2.211 (1.701–2.619)	1.979 (1.700–2.600)	1.903 (1.680–2.381)	2.145 (1.863–2.946)	1.979 (1.739–2.600)
NK ($CD3^-CD16^+CD56^+$), %	11.75 (9.00–15.63)	10.87 (6.65–13.93)	10.70 (9.56–14.72)	13.80 (12.00–16.37)	12.00 (7.49–15.63)
NK ($CD3^-CD16^+CD56^+$), ×10⁹/L	0.266 (0.163–0.350)	0.237 (0.117–0.353)	0.195 (0.161–0.349)	0.302 (0.223–0.417)	0.269 (0.160–0.353)
$CD3^-CD16^+CD56^+CD8^-CD38^-$, % of NK	52.39 (17.65–65.45)	42.95 (14.98–66.46)	12.36 (7.83–64.86)	22.11 (4.94–53.73)	26.44 (10.13–64.94)
$CD3^-CD16^+CD56^+CD8^-CD38^-$, ×10⁹/L	0.111 (0.040–0.224)	0.045 (0.031–0.102)	0.020 (0.014–0.046)	0.061 (0.015–0.166)	0.043 (0.015–0.114)
$CD3^-CD16^+CD56^+CD8^+CD38^+$, % of NK	7.13 (2.75–20.00)	2.99 (0–18.92)	10.84 (2.60–30.50)	12.15 (2.03–32.86)	7.10 (1.16–21.90)
$CD3^-CD16^+CD56^+CD8^+CD38^+$, ×10⁹/L	0.011 (0.005–0.036)	0.006 (0–0.045)	0.021 (0.001–0.049)	0.036 (0.009–0.049)	0.021 (0.001–0.049)
$CD3^-CD16^+CD56^+CD8^-CD38^+$, % of NK	22.00 (9.27–44.51)	18.07 (4.83–50.64)	8.44 (0.78–29.03)	21.28 (2.85–47.14)	18.82 (4.83–47.14)
$CD3^-CD16^+CD56^+CD8^-CD38^+$, ×10⁹/L	0.077 (0.015–0.085)	0.046 (0.014–0.154)	0.010 (0.002–0.085)	0.066 (0.011–0.099)	0.063 (0.008–0.101)
$CD3^-CD16^+CD56^+CD8^+CD38^-$, % of NK	9.14 (4.58–18.15)	4.19 (2.48–34.05)	18.94 (2.41–38.74)	5.21 (1.23–22.67)	5.44 (2.41–34.05)
$CD3^-CD16^+CD56^+CD8^+CD38^-$, ×10⁹/L	0.015 (0.008–0.042)	0.010 (0.006–0.024)	0.010 (0.005–0.056)	0.012 (0.003–0.070)	0.010 (0.005–0.056)

Table prepared by the authors using their own data

Notes:
1 — The data are presented as median (25th–75th percentiles).
2 — p — confidence probability of differences relative to the comparison group (Mann–Whitney U test).
3 — p^* — confidence probability of differences with respect to the subgroup of people with the highest doses (Mann–Whitney U test).

Despite a decrease in the median number of natural killer cells not expressing CD38 and CD8 molecules and in the absolute $CD38^+$ NK count in chronically exposed people from the main group relative to the comparison group, no statistical significance of differences was established due to the significant scatter of individual values in people from the main group with relatively small sample sizes, confidence intervals of median values overlap.

At the same time, no statistically significant differences were found between the relative and absolute natural killer cells count expressing in various combinations of CD8 and CD38 molecules on the cell membrane in chronically irradiated people from different dose subgroups and in persons from the comparison group.

When examining the dose-effect relationship, weak inverse correlations were found between the dose calculated on RBM and the relative, absolute monocyte count, absolute $CD14^+CD16^-$ and $CD14^+CD16^+$ monocyte count, and relative $CD3^-CD16^+CD56^+CD8^-CD38^-$ cell count. A direct weak correlation was found between the relative NKs and the dose calculated per RBM. The correlations between the studied indices of innate immunity and doses of RBM and TSLO irradiation were not statistically significant. In order to clarify the results of the analysis of the dependence of the studied indicators of innate immunity on radiation doses, the sample of the examined persons will be expanded in future research. In chronically exposed people in the long-term period following the beginning of radiation exposure, no statistically significant correlations were established by the Spearman criterion between the quantitative characteristics of leukocytes, lymphocytes or monocytes, as well as the analyzed subpopulations of monocytes and natural killer cells and such factors of non-radiation nature as age, sex and ethnos, or between the studied indicators of innate immunity and the doses of radiation exposure.

The data presented in Table 4 show a statistically significant positive correlation between age at the time of examination and the proportion of inactivated NKs, as well as a negative correlation between the ethnicity of the subjects and the proportion of $CD14^-CD16^+$ monocyte in the peripheral blood. At the same time, a reliable correlation was established between the gender of the subjects and the relative or absolute cell count in the subpopulation of inactivated NKs and the proportion of NKs simultaneously expressing CD8 and CD38 molecules on the cell membrane. No such correlations were found in people from the comparison group.

The results are preliminary. In future research, the study groups will be enlarged.

DISCUSSION

The results of the study agree with and complement previously obtained results of immunological examinations of chronically exposed people from the Techa River cohort. The main dose-forming radionuclide was osteotropic strontium-90 (^{90}Sr), whose characteristic feature is a long half-life (about 30 years), accumulation in bone tissue, and prolonged impact of IR on the central organ of the immune system and hematopoiesis (RBM). The unique nature of radiation exposure — in particular, the combination of external and internal γ-radiation, mainly β-radiation due to ^{90}Sr — apparently underlies the long-term changes in the immune system in the residents of the Techa River cohort. No changes in the absolute leukocyte count or relative and absolute monocyte content in blood were detected between exposed and unexposed study participants.

The subpopulation composition of monocytes and expression of activation molecules on NK cells in the peripheral blood of people from the Techa River cohort in long-term periods following chronic radiation exposure was investigated for the first time.

Monocyte–macrophages represent a heterogeneous cluster of cells having great plasticity and multiple functions determined by the type of activating signal. They are radioresistant cells whose functions can be modulated by exposure (AI); depending on the radiation dose and fractionation, such cells exert both pro- or anti-inflammatory, as well as pro- or antitumor activity. At present, it is not possible to systematize information on specific radiation-induced modulations of monocyte–macrophages [16].

CD14 molecule, a type of Toll-like receptor (TLR), appears at the early stages of monocyte maturation and is its specific marker [16]. Differentiation of classical $CD14^{++}CD16^-$ monocytes occurs from a medullary myeloid precursor. Outside the BCC they mature into intermediate $CD14^{++}CD16^+$ monocytes, differentiate into non-classical $CD14^+CD16^{++}$ monocytes and further as tissue

Table 4. The results of the correlation analysis between some non-radiation factors and the studied indicators of immunity

Pairs of parameters, units	Comparison group, *n* = 10		Main group, *n* = 33	
	SR	*p*	*SR*	*p*
$CD14^-CD16^+$ monocytes, $\times10^9$/L & Ethnicity	–0.59	0.072	–0.41	0.019
$CD3^-CD16^+CD56^+CD8^-CD38^-$, % of NK & Age at the time of examination	0.42	0.228	0.39	0.036
$CD3^-CD16^+CD56^+CD8^-CD38^-$, % of NK & Gender	0.09	0.811	0.50	0.005
$CD3^-CD16^+CD56^+CD8^-CD38^-$, $\times10^9$/L & Gender	–0.17	0.631	0.46	0.010
$CD3^-CD16^+CD56^+CD8^+CD38^+$, % of NK & Gender	0.17	0.631	0.36	0.050

Table prepared by the authors using their own data

Notes: *SR* — Spearman correlation coefficient, *p* — Confidence probability

macrophages and dendrocytes perform remodeling and repair of damaged tissues [17, 18]. Late maturation of peripheral monocytes is accompanied by the expression of FcγRIII (CD16) on the cell membrane with a simultaneous decrease in the number of CD14 molecules. At the same time, CD16 expression is associated with an increased ability of monocytes to present antigen, which increases with the loss of CD14 expression on the cell membrane [7]. The relatively low phagocytic activity and enhanced ability to produce cytokines and antigen presentation in such cells is due to higher expression of major histocompatibility complex class II (MHC II) molecules [19].

Monocyte subpopulations perform different functions, which are determined either by the state of their microenvironment representing a complex cytokine-cell system, or by their linear differentiation [18]. Functionally, all monocytes/macrophages are conventionally classified into two types: proinflammatory (M1), which mainly produce IL-18, IL-12, IL-26 and thus stimulate Th1 and Th17 responses, and anti-inflammatory (M2), which mainly produce IL-10 and transforming growth factor beta (TGF-β) and participate in immunoregulation and tissue repair. There are many intermediate and transitional forms between these two clusters of cells, and a dynamic balance of cytokines is maintained depending on the current needs of the macroorganism [7].

The ability of macrophages to respond to various endogenous and exogenous pro- and anti-inflammatory stimuli ensures a high heterogeneity of their phenotype. IFN-γ activates pro-inflammatory macrophages (M1), which participate in the Th1-dependent immune response to oncotransformed target cells. Anti-inflammatory macrophages (M2) are involved in Th2-dependent immune responses, repair processes, and the pathogenesis of some tumors. The spectrum of cytokines produced by M2 cells includes IL-1, IL-6, IL-10, vascular endothelial growth factor (VEGF), and TGF-β, which under certain conditions provide proliferation and metastasis of tumor cells. The subpopulation of monocytes with the $CD14^{low}CD16^{bright}$ phenotype "non-classical monocytes" corresponds to anti-inflammatory cells [7].

Normal monocytes/macrophages play an important role in antitumor immune surveillance by antigen-mediated activation of T-cytotoxic cells or direct lysis of tumor cells in the activated state. Activated macrophages exhibit antitumor activity due to lysing enzymes, TNF-α synthesis and free radical production. Intermediate monocytes in comparison with monocytes of other subpopulations are the main producers of IL-1β, IL-6 and TNF-α, have the greatest ability to perform transendothelial migration and formation of reactive oxygen species [20, 21].

Oncotransformed cells are characterized by reduced expression of major histocompatibility complex class I molecules, which modifies inhibitory signals from other NK receptors. Activating NK receptors interact with stress-inducible proteins that are expressed by tumor cells. The secretory process is triggered in NK cells representing cytoplasmic vesicles containing serine esterases (granzymes A and B) are released by local exocytosis into the space between the effector cell and the target. In NK, a specialized mechanism of transformed cell killing is associated with perforin contained within the granules, which has lytic activity against target cells. Immediately after lymphocyte binding to the target cell, pores are formed in its membrane, NK granules are exocytosed and their contents — granzymes and perforin — are released. Further, a cascade of lytic processes is triggered in the target cell, which leads to deoxyribonucleic acid (DNA) degradation and subsequent cell death. The ability of NK to synthesize cytokines — primarily IFN-γ — determines their participation in the regulation of other parts of antitumor immunity [11].

The scientific literature discusses the hypothesis that in the long-term period following exposure of the organism to IR, senescent cells (primarily leukocytes and macrophages, as well as fibroblasts and others) are one of the main and constant sources of reactive oxygen species and reactive nitrogen species in tissues. These contribute to the maintenance of a high level of free radicals therein and can lead to damage of cells and subcellular structures up to fibrosis and neoplastic transformation [22, 23].

It should be emphasized that the revealed features of immune status, which are more pronounced in chronically exposed people with maximum absorbed doses calculated per RBM, were registered during the period of realization of carcinogenic effects of irradiation and may play a certain role in their development. The results of correlation analysis of the influence of factors of non-radiation nature in people from the studied groups require considerable caution in interpretation due to the relatively small sample size, a factor that introduces significant uncertainty in the assessment of pairwise correlations.

CONCLUSION

The study revealed statistically significant changes in monocyte subpopulations in people exposed to chronic radiation. In particular, in persons irradiated in the dose range of 250 and up to 699 mGy, the relative $CD14^-CD16^+$ monocyte count was significantly higher than in the comparison group; their absolute count exceeded the similar index in persons from the third subgroup with a maximal radiation burden. The preliminary results obtained in practically healthy exposed people from the Techa River cohort may indicate some latent tension of regulatory mechanisms in the immune system — in particular, the monocyte–macrophage systems and NK, which act according to the feedback principle and are aimed at compensating proinflammatory immune shifts [23] — that are to a lesser extent expressed in non-exposed people.

In all examined people from the main group and the comparison group, the absolute leukocyte count, relative and absolute monocyte count, as well as the relative and absolute monocyte with phenotypes $CD14^+CD16^-$, $CD14^+CD16^+$, $CD14^-CD16^+$ count, did not differ statistically significantly. Relative and absolute amounts of NK cells expressing in various combinations of CD8 and CD38 molecules on the cell membrane did not differ statistically significantly between chronically exposed people and those from the comparison group.

No statistically significant Spearman correlations were found between the studied indices of innate immunity and factors of radiation nature in chronically irradiated people.

The study of subpopulations of monocytes and natural killer cells in peripheral blood in chronically exposed population of the Techa River embankment zone will be continued.

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