# SUBPOPULATION COMPOSITION OF T-HELPERS IN THE PERIPHERAL BLOOD OF PERSONS CHRONICALLY EXPOSED TO RADIATION IN THE LONG TERM

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Earlier, it has been convincingly established that exposure to ionizing radiation (IR) alters the T cell-mediated immunity in the long term. However, a search for papers describing the effect chronic exposure to radiation has on various subpopulations of T-helpers yielded no results. Therefore, we designed this study seeking to investigate the quantitative characteristics of various subpopulations of T-helpers in the peripheral blood of individuals chronically exposed to low-level radiation for a long period of time. The study involved 102 chronically exposed Techa Riverside residents (Russia) aged 60–87 years. The participants were divided into two groups, one comprised of exposed individuals with the average red bone marrow (RBM) irradiation dose of 567 ± 73 mGy, another, the control group, comprised of people with the irradiation dose below 70 mGy. With the help of flow cytometry, we identified the quantitative characteristics of T-helper subpopulations in the peripheral blood at various stages of their differentiation, as well as various T-helper subpopulations of central and effector memory. The study revealed no significant differences in the composition of T-helper subpopulations in the compared groups. We discovered a significant growth of the double positive follicular T-helper 17 subpopulation in the population of central memory T-helpers, which is associated with the increase of RBM ($p = 0.04$; $S = 0.19$), thymus and peripheral lymphoid organs ($p = 0.03$; $S = 0.22$) irradiation dose. In the group of exposed individuals, the number of naive T-helpers ($p = 0.009$) and double positive follicular T-helpers 17 in the $T_{EM}$ subpopulation ($p = 0.04$) was decreasing as the age of participants increased, and the number of effector memory T-helpers, on the contrary, increased with age ($p = 0.04$). We have not registered similar phenomena in the comparison group.

**Keywords:** T-helpers, follicular T-helpers, immunity, chronic exposure

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# СУБПОПУЛЯЦИОННЫЙ СОСТАВ Т-ХЕЛПЕРОВ В ПЕРИФЕРИЧЕСКОЙ КРОВИ ХРОНИЧЕСКИ ОБЛУЧЕННЫХ ЛИЦ В ОТДАЛЕННОМ ПЕРИОДЕ

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К настоящему времени убедительно показано, что воздействие ионизирующего излучения (ИИ) вызывает долгосрочное изменение Т-клеточного иммунитета. Однако количественных исследований влияния хронического радиационного воздействия на различные субпопуляции Т-хелперов в доступной литературе найдено не было. Поэтому целью работы было исследовать количественные характеристики различных субпопуляций Т-хелперов в периферической крови лиц, подвергшихся хроническому низкоинтенсивному радиационному воздействию, в отдаленные сроки после начала облучения. В исследовании приняло участие 102 хронически облученных жителя прибрежных сел реки Течи (Россия) в возрасте 60–87 лет, которые были подразделены на облученных лиц (средняя накопленная доза облучения красного костного мозга составила 567 ± 73 мГр) и группу сравнения (доза облучения не превышала 70 мГр). Методом проточной цитометрии определяли количественные характеристики субпопуляций Т-хелперов в периферической крови на разной стадии их дифференцировки, а также различные субпопуляции Т-хелперов центральной и эффекторной памяти. В ходе исследования не выявлено статистически значимых различий в субпопуляционном составе Т-хелперов в сравниваемых группах. Отмечено статистически значимое повышение содержания субпопуляции «double positive» фолликулярных Т-хелперов 17 в популяции Т-хелперов центральной памяти с увеличением дозы облучения ККМ ($p = 0,04$; $S = 0,19$), а также тимуса и периферических лимфоидных органов ($p = 0,03$; $S = 0,22$). В группе облученных лиц зарегистрировано снижение количества наивных Т-хелперов ($p = 0,009$) и «double positive» фолликулярных Т-хелперов 17 в составе субпопуляции $T_{EM}$ ($p = 0,04$) и увеличение количества Т-хелперов эффекторной памяти ($p = 0,04$) с увеличением возраста, чего не наблюдалось в группе сравнения.

**Ключевые слова:** Т-хелперы, фолликулярные Т-хелперы, иммунитет, хроническое облучение

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Earlier, it has been convincingly established that exposure to ionizing radiation (IR) alters the T cell-mediated immunity in the long term. A number of studies have identified that irradiated individuals, including Techa Riverside residents, have more mutations in the genes of the T cell receptor (TCR mutations). An in-depth analysis of the immune status of such people allowed establishing the special role cytotoxic $CD3^+CD16^+CD56^+$ lymphocytes play in the elimination of TCR mutant cells [1].

Regarding the impact of IR on T cell populations, there are data showing changes in the peripheral blood T helper indices. For example, 20 years after exposure, survivors of the Hiroshima and Nagasaki atomic bombing that received the dose greater than 1 Gy had the number of naive T helpers ($CD4^+CD45RA^+$ phenotype) decreasing [2]. The Chernobyl accident victims exhibited similar reactions: those who received high doses of radiation had less T-helpers in the peripheral blood [3]. A study that involved employees of Mayak Production Association (a nuclear industry production facility) has revealed a linear dose-dependent decrease in the number of peripheral blood T helpers [4]. A study of low dose occupational exposure that involved employees of Kozloduy, a Bulgarian nuclear power plant, yielded an assumption about a possible immune response shift from $Th_1$ to $Th_2$ [5]. However, a search for quantitative studies describing the effect chronic exposure to radiation has on various subpopulations of T helpers, including $Th_{17}$, $Th_{22}$, $Th_9$ etc., yielded no results.

This study aimed to investigate the quantitative characteristics of various subpopulations of T helpers in the peripheral blood of individuals chronically exposed to low-level radiation for a long period of time.

## METHODS

The study of T helper subpopulations involved 102 people whose irradiation conditions and nature were described in detail earlier [6]. Techa Riverside residents were exposed to gamma radiation both externally and internally, mainly from $^{90}Sr$ and $^{137}Cs$. The contamination came from the liquid radioactive waste discharged into the river by Mayak. The largest doses were absorbed by the red bone marrow (RBM) in 1950s–1960s; the associated key source of radiation was $^{90}Sr$. We divided the chronically exposed people into two groups: exposed individuals ($n = 54$) and the control group ($n = 48$). It is important to note that currently, among the Techa Riverside residents, people with the accumulated radiation dose exceeding 100 mGy (localization — stomach and RBM) suffer and die from malignant neoplasms, including leukemia, more often [6].

The study inclusion criteria were: permanent residence in one of the 41 Techa River villages from 01.01.1950 to 12.31.1960; availability of data on the established doses accumulated in the RBM, thymus and peripheral lymphoid organs (as measured with the TRDST 2016 dosimetric system) [7]; absence of autoimmune, oncological, acute or chronic (exacerbating) inflammatory diseases, hemoblastoses, renal or hepatic insufficiency, acute cerebrovascular accidents in the previous three months; no intake of antibiotics, glucocorticoids and cytostatics in the previous six months; no X-ray examinations in the previous six months.

The control group included people living in the similar socio-economic conditions whose RBM radiation dose accumulated over their entire lives did not exceed 70 mGy [8] (Table 1).

For the study, we sampled 9 ml of fasting blood of the participants into vacuum tubes filled with K3-EDTA (Greiner Bio-One; Austria). Flow cytometry enabled assessment of the relative number of T helper subpopulations by the level of expression of CD45RA (naive T helpers marker), CD62L (marker of directed cell migration to the peripheral lymphoid organs), CCR4, CCR6, CXCR3, and CXCR5. Into the flow cytometer (Beckman Coulter; USA) tube, we added 100 µl of the test sample, 5 µl of CD3 and CD4 monoclonal antibodies (Beckman Coulter, USA; conjugated with Krome Orange and Pacific Blue, respectively), 20 µl of CD45RA and CD62L monoclonal antibodies (Beckman Coulter, USA; conjugated with PE and FITC, respectively), 5 µl of monoclonal antibodies CCR4, CCR6, CXCR3 and CXCR5 (Beckman Coulter, USA; conjugated with APC, Per-CP-eFluorTM710, PE-Cyanine7 and PE-eFluor® 610 respectively). The samples were incubated for 20 minutes in a dark place at room temperature. Then, we added 1 ml of VersaLyse Lysing Solution (Beckman Coulter; USA) to the tube to remove erythrocytes, and then left the samples to incubate for 10 more minutes under the same conditions. After incubation, the samples were analyzed in a Navios flow cytometer (Beckman Coulter; USA).

The gating tactic we employed in the context of T helper subpopulations analysis relied on the identification of T helpers by the presence of CD3 and CD4 markers on the cell surface. Next, we divided the population of $CD3^+CD4^+$ cells into subpopulations of T helpers at different stages of differentiation by the presence of surface markers CD45RA and CD62L. Namely, we differentiated between phenotype $CD3^+CD4^+CD45RA^+CD62L^+$ naive T helpers ($T_{Naive}$), central memory phenotype $CD3^+CD4^+CD45RA^-CD62L^+$ T helpers ($T_{CM}$), effector memory phenotype $CD3^+CD4^+CD45RA^-CD62L^-$ T helpers ($T_{EM}$) and terminally differentiated phenotype

**Table 1.** Characteristics of the studied groups

| Group characteristics | | Exposed individuals | Control group |
|---|---|---|---|
| | | $n = 54$ | $n = 48$ |
| Age at the time of examination, years, M ± SE (min–max) | | 73.26 ± 0.58 (67–84) | 68.73 ± 0.96 (60 –87) |
| Gender, person (%) | male | 22 (40.7) | 17 (35.4) |
| | female | 32 (59.3) | 31 (64.6) |
| Ethnicity, people (%) | Slavs | 17 (31.5) | 21 (43.8) |
| | Turks | 35 (64.8) | 26 (54.2) |
| | Not established | 2 (3.7) | 1 (2) |
| Dose accumulated in the RBM, mGy, M ± SE (min–max) | | 567 ± 73 (80.20–2930) | 17.20 ± 2.25 (1.89–55) |
| Dose accumulated in the thymus and peripheral lymphoid organs, mGy, M ± SE (min–max) | | 79.80 ± 10.70 (4.63–355) | 7.35 ± 1.29 (0.21–39.5) |

**Note:** *n* — the number of subjects; M ± SE — mean ± standard error.

**Table 2.** Phenotype characteristics of the studied T helper subpopulations, peripheral blood of exposed individuals

| Cell phenotype | Population name |
|---|---|
| $CXCR5^-CXCR3^-CCR6^-CCR4^+$ | T helpers 2 ($Th_2$) |
| $CXCR5^-CXCR3^-CCR6^+CCR4^-$ | T helpers 17 ($Th_{17}$) |
| $CXCR5^-CXCR3^-CCR6^+CCR4^+$ | T helpers 17 and T helpers 22 ($Th_{17}$ and $Th_{22}$) |
| $CXCR5^-CXCR3^+CCR6^-CCR4^-$ | T helpers 1 and T helpers 9 ($Th_1$ and $Th_9$) |
| $CXCR5^-CXCR3^+CCR6^+CCR4^-$ | "Non-classical" Th17 ($Th_1/Th_{17}$) |
| $CXCR5^+CXCR3^-CCR6^-CCR4^-$ | Follicular T helpers 2 ($Tfh_2$) |
| $CXCR5^+CXCR3^-CCR6^-CCR4^+$ | Follicular T helpers 2 ($Tfh_2$) |
| $CXCR5^+CXCR3^-CCR6^+CCR4^-$ | Follicular T helpers 17 double negative (double negative $Tfh_{17}$) |
| $CXCR5^+CXCR3^-CCR6^+CCR4^+$ | Follicular T-helpers 17 ($Tfh_{17}$) |
| $CXCR5^+CXCR3^+CCR6^-CCR4^-$ | Follicular T helpers 1 ($Tfh_1$) |
| $CXCR5^+CXCR3^+CCR6^+CCR4^+$ | Follicular T helpers 17 double positive (double positive $Tfh_{17}$) |

$CD3^+CD4^+CD45RA^+CD62L^-$ T helpers (TEMRA). In the $T_{CM}$ and $T_{EM}$ populations, subpopulations of various T helpers were identified by the presence of CCR4, CCR6, CXCR3, and CXCR5 markers [9–11] (Table 2).

For statistical data processing we employed SigmaPlot software (SYSTAT Software; USA). Kolmogorov-Smirnov test enabled verification of normalcy of distribution of the indicators. To compare the arrays of nonparametric data, we applied the Mann–Whitney U-test. First of all, the above parameters were evaluated for T helpers at different levels of differentiation, then we processed the data describing subpopulations of central and effector memory T helpers.

To identify dependencies, we used the Spearman's rank correlation coefficient and the Pearson correlation coefficient, as well as linear regression. The results were considered reliable at 5% significance level.

## RESULTS

The currently adopted approach is to rely on CD markers expression to identify T helpers at different stages of differentiation. The literature offers detailed descriptions of the ability of TNaive not differentiated in the secondary lymphoid organs (antigen-dependent differentiation) to give rise to memory T cells and effector cells. $T_{CM}$s carry the CD62L adhesion molecule, which determines their ability to largely localize in the secondary lymphoid organs. $T_{EM}$s are not able to penetrate into the peripheral lymphoid organs, however, they carry a wide range of different adhesion and chemokine molecules on their surface, which aid their migration into tissues and organs. The ability of $T_{EM}$s to proliferate and differentiate is reduced, and the cells part of this population are the main producers of effector cytokines, such as IFNγ and IL4. TEMRA effector cells are considered to be the final stage of T lymphocyte differentiation process in the peripheral blood. The effector properties of TEMRA require no costimulation; they manifest under the action of cytokines produced by the inflamed tissue [12].

At the first stage of the study, we relied on the expression of CD45RA (surface marker) and CD62L (marker of directed cell migration to the peripheral lymphoid organs) to investigate the relative number of T helpers at different stages of differentiation: $T_{Naive}$, $T_{CM}$, $T_{EM}$, and TEMRA (Table 3).

Comparison of the quantitative indicators of lymphocytes and T helpers at different stages of differentiation in the exposed and control groups revealed no significant differences (Table 3).

The expression of CCR4, CCR6, CXCR3, and CXCR5 chemokine receptors allowed estimating the number of T helper populations in the $T_{CM}$ and $T_{EM}$ subpopulations. The $Th_1$, $Th_2$, $Th_{17}$ and $Th_{22}$, $Th_9$ subpopulations, as well as follicular T helpers, are predominantly found in the $T_{CM}$ and $T_{EM}$ populations. All these cells have unique developmental and regulatory pathways and play different roles in the immunity and immunity-mediated pathologies [13].

Tables 4 and 5 present the results of estimation of representation of various subpopulations of T helpers in the $T_{CM}$ and $T_{EM}$ populations, as well as the ratio of $Th_1/Th_2$ and $Th_1/Th_{17}$ in the exposed individuals group.

We discovered no significant differences in the $T_{CM}$ population T helper subpopulation values between the exposed individuals and control groups.

**Table 3.** The number of T helpers of different subpopulations in the compared groups

| Indicator | Exposed individuals | Control group |
|---|---|---|
| | Me ($Q_1$–$Q_2$) | |
| Lymphocytes, abs. | 2.17 (1.49–2.76) | 2.07 (1.62–2.55) |
| Lymphocytes, % | 32.00 (22.00–36.80) | 31.10 (26.00–38.75) |
| $CD3^+CD4^+$-cells, % | 36.34 (31.83–41.54) | 39.50 (33.15–45.22) |
| $T_{Naive}$, % | 26.04 (14.89–39.66) | 27.79 (17.46–36.77) |
| $T_{CM}$, % | 36.83 (30.23–42.65) | 36.74 (31.87–43.48) |
| $T_{EM}$, % | 27.85 (20.35–38.58) | 30.63 (23.24–38.09) |
| TEMRA, % | 2.37 (1.15–4.18) | 1.83 (1.22–5.72) |

**Table 4.** Relative number (%) of $T_{CM}$ population T helpers (data for the study participants)

| Indicator, % | Exposed individuals | Control group |
|---|---|---|
| | Me ($Q_1$–$Q_2$) | |
| $Th_2$ | 2.51 (0.99–5.16) | 4.33 (2.54–6.63) |
| $Th_{17}$ | 2.43 (0.92–3.64) | 2.58 (1.38–4.19) |
| $Th_{17}$ и $Th_{22}$ | 0.23 (0.07–0.75) | 0.40 (0.07–0.97) |
| $Th_1$ и $Th_9$ | 32.79 (24.65–37.70) | 33.03 (25.03–37.43) |
| $Th_1/Th_{17}$ | 1.28 (0.58–2.61) | 1.41 (0.36–3.05) |
| $CXCR5^+CXCR3^-CCR6^-CCR4^-$, $Tfh_2$ | 8.46 (5.69–11.11) | 8.03 (5.29–10.68) |
| $CXCR5^+CXCR3^-CCR6^-CCR4^+$, $Tfh_2$ | 0.13 (0.05–0.39) | 0.13 (0.06–0.31) |
| double negative $Tfh_{17}$ | 5.98 (3.26–9.67) | 7.56 (4.29–11.32) |
| $Tfh_{17}$ | 0.14 (0.04–0.34) | 0.21 (0.05–0.53) |
| $Tfh_1$ | 6.11 (4.07–8.92) | 6.54 (4.98–8.14) |
| double positive $Tfh_{17}$ | 0.05 (0.00–0.16) | 0.04 (0.00–0.08) |
| $Th_1/Th_2$ | 11.70 (6.24–32.08) | 7.73 (3.60–15.97) |
| $Th_1/Th_{17}$ | 13.43 (7.65–38.59) | 11.63 (7.51–22.32) |

Comparison of the $T_{EM}$ populations' T helper subpopulation indicators revealed no significant differences between the study groups.

To establish the long-term dependence of the number of different subpopulations of T helpers at different stages of differentiation in the peripheral blood of the exposed individuals we applied the Spearman's rank correlation coefficient and the Pearson correlation coefficient. "Long-term" here means that RBM, thymus, and peripheral lymphoid organs have accumulated the dose a long time ago. The analysis procedure covered both groups.

The correlation analysis did not reveal significant associations of the number of T helpers at different stages of differentiation in the peripheral blood of the exposed individuals with the dose accumulated by RBM, thymus and peripheral lymphoid organs.

Investigating the dependence of content of various $T_{CM}$ population's T helper subpopulations on the radiation dose accumulated by the RBM, thymus and peripheral lymphoid organs, we discovered that the content of double positive $Tfh_{17}$ subpopulation depended significantly on the degree of irradiation of the thymus and peripheral lymphoid organs ($p$ = 0.02; $S$ = 0.23). However, linear regression analysis did not reveal significant dose-based dependences. As for the remaining studied subpopulations of T helpers, we also discovered no significant dependences on the dose values.

A similar analysis was carried out to investigate the dependences of the content of various $T_{EM}$ population's T helper subpopulations on the degree of irradiation of RBM, thymus and peripheral lymphoid organs. No statistically significant dependences of the studied parameters of the $T_{EM}$ populations on the dose values were found.

It is known that with age, immune system of the human beings undergoes involutional changes: the number of some subpopulations of T helpers goes down [14], the direction of differentiation changes [15], and their functioning is disrupted [16]. With this in mind, we have also investigated dependence of the content of various peripheral blood T helper subpopulations on age (Table 6; the age was that reached by the participants at the time of the study).

Correlation analysis revealed a significant association between the decrease in the number of of $T_{Naive}$ and age in the exposed group. The values were $p$ = 0.009, $S$ = –0.35 and $p$ = 0.01, $R$ = –0.34 (Spearman's rank correlation coefficient and Pearson correlation coefficient, respectively). No such association was registered in the control group. In the exposed group, we have also found that the number of $T_{EM}$ grows up significantly with age ($p$ = 0.04, $S$ = 0.28 and $p$ = 0.02, $R$ = 0.33,

**Table 5.** Relative number (%) of TEM population T helpers (data for the study participants)

| Indicator, % | Exposed individuals | Control group |
|---|---|---|
| | Me ($Q_1$–$Q_2$) | |
| $Th_2$ | 0.67 (0.26–1.52) | 0.94 (0.34–2.30) |
| $Th_{17}$ | 4.29 (2.37–6.80) | 5.22 (3.17–6.82) |
| $Th_{17}$ и $Th_{22}$ | 0.23 (0.03–0.52) | 0.17 (0.05–0.59) |
| $Th_1$ и $Th_9$ | 45.96 (39.49–57.57) | 51.51 (36.74–56.46) |
| $Th_1/Th_{17}$ | 2.52 (1.08–5.52) | 3.34 (1.35–7.23) |
| $CXCR5^+CXCR3^-CCR6^-CCR4^-$, $Tfh_2$ | 2.29 (1.59–3.33) | 2.22 (1.18–7.23) |
| $CXCR5^+CXCR3^-CCR6^-CCR4^+$, $Tfh_2$ | 0.02 (0–0.09) | 0 (0–0.04) |
| double negative $Tfh_{17}$ | 2.11 (1.24–3.45) | 2.75 (1.55–4.35) |
| $Tfh_{17}$ | 0.02 (0–0.10) | 0.04 (0–0.15) |
| $Tfh_1$ | 2.48 (1.49–3.87) | 2.42 (1.84–3.62) |
| double positive $Tfh_{17}$ | 0 (0–0.03) | 0 (0–0.02) |
| $Th_1/Th_2$ | 62.63 (26.95–224.95) | 44.66 (20.59–111.95) |
| $Th_1/Th_{17}$ | 10.19 (6.13–21.56) | 9.92 (6.15–15.25) |

**Table 6.** Dependence of indicators of various subpopulations of T helpers on the age of participants (as of the time of the study)

| Indicator, % | Exposed individuals | | Control group | |
|---|---|---|---|---|
| | *S* (*p*) | *R* (*p*) | *S* (*p*) | *R* (*p*) |
| $T_{Naive}$ | –0.35 (0.009) | –0.34 (0.01) | – | – |
| $T_{EM}$ | 0.28 (0.04) | 0.33 (0.02) | – | – |
| Central memory T helpers | | | | |
| double positive $Tfh_{17}$ | – | – | – | 0.32 (0.03) |
| Effector memory T helpers | | | | |
| double positive $Tfh_{17}$ | –0.28 (0.04) | – | 0.37 (0.01) | 0.39 (0.006) |

**Note:** *S* (*p*) — the Spearman's rank correlation coefficient (correlation significance level); *R* (*p*) — Pearson linear correlation coefficient (correlation significance level).

Spearman's rank correlation coefficient and Pearson correlation coefficient, respectively). Nothing similar was discovered in the control group. These dependencies were investigated with a linear regression analysis (Figure 1; moving average graphs).

We discovered a significant dependence of the content of $T_{EM}$ subpopulation's double positive $Tfh_{17}$ (peripheral blood) on age in both groups, but these associations were multidirectional: in the exposed individuals, the number of cells of this population decreased significantly with age ($p = 0.04$; $S = –0.28$), and in the control group, on the contrary, the said number increased ($p = 0.003$, $S = 0.32$ and $p = 0.05$, $R = 0.29$, Spearman's rank correlation coefficient and Pearson correlation coefficient, respectively). Moreover, in the control group we revealed an age-dependent increase in the $T_{CM}$ subpopulation's double positive $Tfh_{17}$ counts ($p = 0.009$, $R = 0.38$), while in the exposed group no such dependence was registered.

Linear regression analysis of the dependence of number of $T_{CM}$ and $T_{EM}$ subpopulations' double positive $Tfh_{17}$ on the age of the exposed group participant revealed no significant correlations; for the control group, the results are shown on Fig. 2 (moving average graph).

The analysis of dependence of the content of various peripheral blood T helper subpopulations on the age of exposed individuals at the beginning of exposure showed the following correlations: the amount of $T_{Naive}$ decreased as the age increased ($p = 0.03$, $S = –0.34$ and $p = 0.04$; $R = –0.32$, Spearman's rank correlation coefficient and Pearson correlation coefficient, respectively), while the number of $T_{EM}$s increased ($p = 0.03$, $S = 0.35$ and $p = 0.04$, $R = 0.32$, Spearman's rank correlation coefficient and Pearson correlation coefficient, respectively) (Fig. 3).

As for the remaining T helper populations, we discovered no significant dependences on the age reached at the time of the study and at the beginning of exposure.

## DISCUSSION

T helper cells are critically important to the regulation of immune system: the range of their actions stretches from activating B-lymphocytes, cytotoxic T-lymphocytes and other cell populations to suppressing immune response.

However, in addition to supporting the functions of adaptive immunity, T helpers can also be involved in the development of autoimmune [17, 18] and oncological diseases [19, 20]. The development of chronic lymphocytic leukemia was reported to be associated with the spread of abnormal follicular T helper cells that elevate levels of cytokines and produce costimulatory factors that promote tumor cell proliferation [21]. Other studies [22, 23] have shown protumor activity of $Th_{17}$, which manifests in the production of immunosuppressive cytokines and chemokines in the tumor microenvironment, thus stimulating its growth and metastasis.

The balance of various subpopulations of T helpers plays an important role in the immune response. For example, cytokines produced by $Th_2$ cells block production of $Th_1$ cytokines and are their natural killer cells. In addition, Th1 cells can inhibit the differentiation and proliferation of basophils and eosinophils, the activity of which is controlled by the synthesis of $Th_2$ cytokines [24]. Ionizing radiation compromises the $Th_1/Th_2$ immune balance and tilts it towards $Th_2$ dominance, the unbalanced state potentially contributing to immune system dysfunction after exposure [25]. A number of studies report a shift in the $Th_1/Th_2$ balance towards $Th_2$ in the cases of hematological

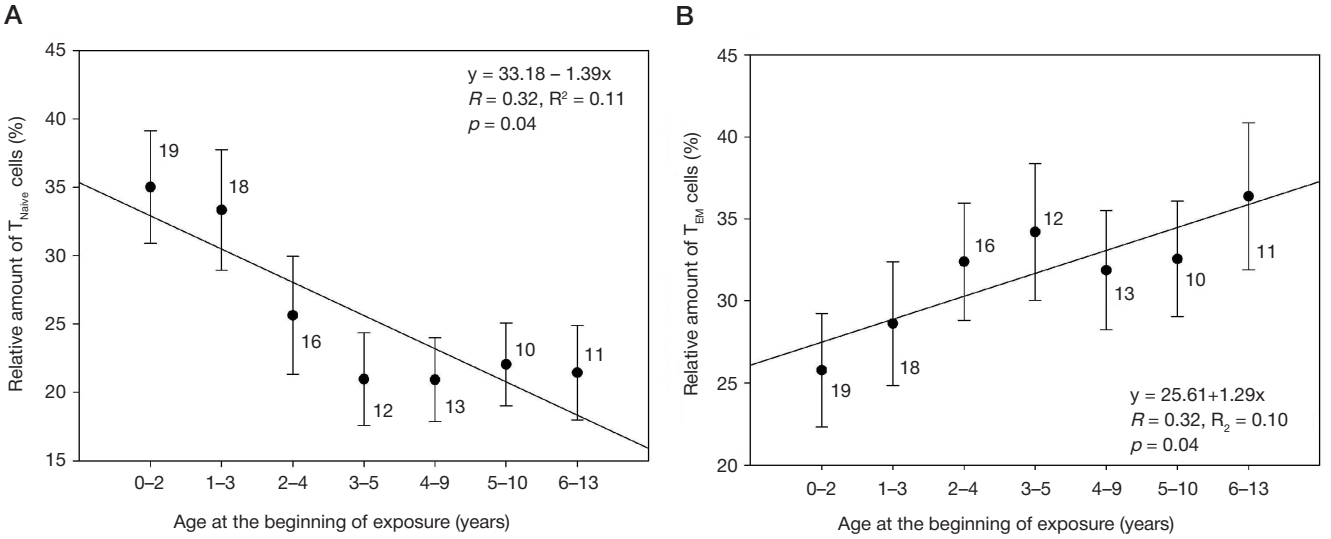


**Fig. 1.** Dependence of the relative amounts of peripheral blood TNaive (**A**) and TEM (**B**) on age, exposed individuals; linear regression

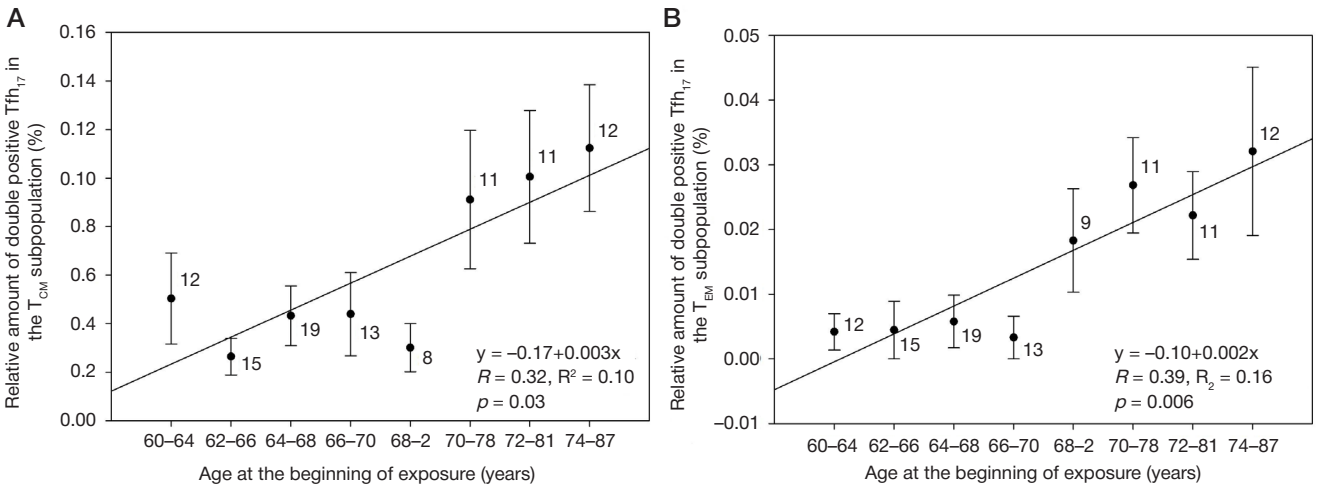


**Fig. 2.** Dependence of the content of double positive $Tfh_{17}$ in $T_{CM}$ (**A**) and $T_{EM}$ (**B**) subpopulations (peripheral blood) on age (as of the time of the study), control group; linear regression

malignant neoplasms [26–28]. More recent papers describe another association of immunocompetent cells, $Th_1/Th_{17}$, which, when unbalanced, contributes to the development of autoimmune diseases, primarily rheumatoid arthritis [29, 30].

Investigating the long-term effects of exposure to radiation, we discovered no significant differences in the relative content of various peripheral blood T helper subpopulations, as well as the $Th_1/Th_2$ and $Th_1/Th_{17}$ associations, between the exposed individuals and control groups. However, in the exposed group, we did reveal some peculiarities (differences from the control group indicator values) in the dependence of individual populations of peripheral blood T helpers on the degree of irradiation of thymus, peripheral lymphoid organs and age.

In the exposed group, we established that the increase in the amount of peripheral blood $T_{CM}$ population's double positive $Tfh_{17}$ depends on the dose accumulated by the thymus and peripheral lymphoid organs. Other studies that involved people exposed to radiation have also registered dose-dependent changes in the number of T helpers and their functional properties. Thus, the Hiroshima and Nagasaki atomic bombing survivors exhibited a dose-dependent decrease in the number of $CD4^+$ T cells in peripheral blood [3], a higher occurrence of T cell receptor mutations (mainly in $CD4^+$ T cells population) [31], and a dose-dependent shortening of the T helper telomere length at doses above 0.5 Gy [32]. In Mayak employees that were chronically exposed to radiation the T helper part of the immune system was also changed: a greater dose (2–4 Gy, external irradiation) translated into a smaller number of $CD4^+$ cells [33]; the concentrations of some cytokines and chemokines changed, too. The results obtained allowed a conclusion that the identified changes in the parameters of immune systems of the examined individuals supported chronic inflammatory status and could contribute to the development of late radiation-induced pathologies, such as cardiovascular and malignant diseases [5, 34].

In addition to the dependences on radiation dose, age-related changes were found in the groups. We discovered that the number of $T_{Naive}$ in the peripheral blood of the exposed individuals decreased with age, and the amount of $T_{EM}$s increased, the latter capable of migrating through the vascular and tissue endothelium to inflammation foci and triggering a rapid immune response with the synthesis of predominantly effector cytokines [12]. These results are consistent with the literature data describing the decrease of naive T helper cell numbers with age in people older than 70–75 years [14]. In addition, naive T cells in older people grow prone to effector differentiation [35]. It should be noted that no such correlations were found in the control group, where the number of double positive $Tfh_{17}$ in the $T_{CM}$ and $T_{EM}$ subpopulations increased with age, while in the exposed group, on the contrary, the $T_{EM}$ subpopulation's double positive $Tfh_{17}$ content was growing down very slightly with age. Follicular T helper cells express CXCR5 chemokine receptor, which allows them to migrate to its CXCL13 ligand in the B-cell follicle. Normal follicular T helper

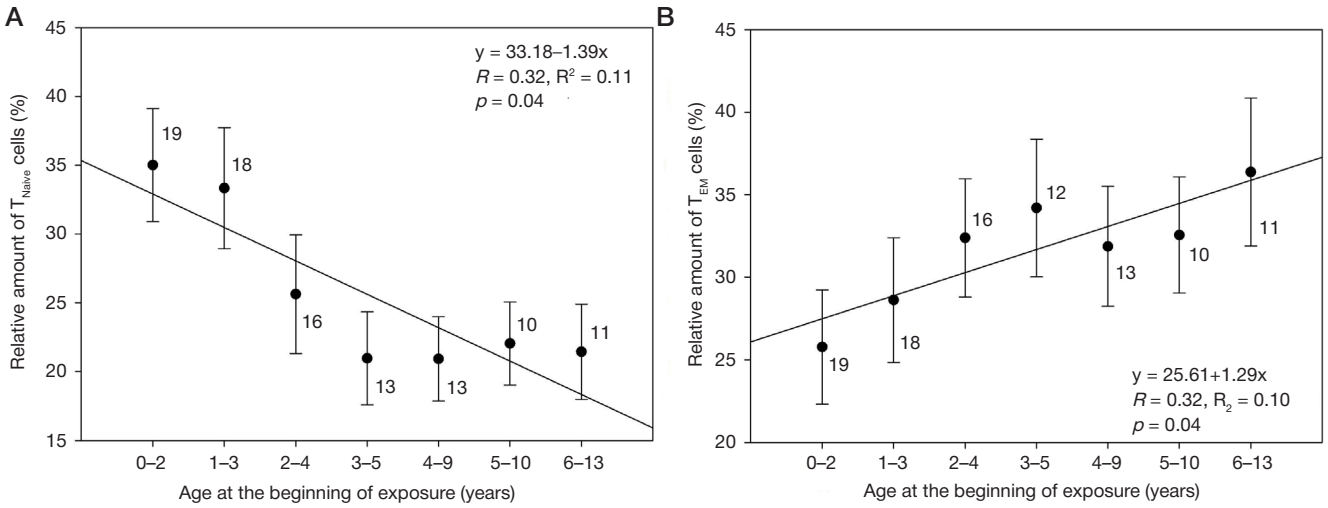


**Fig. 3.** Dependence of the relative amounts of peripheral blood $T_{Naive}$ (**A**) and $T_{EM}$ (**B**) on age, exposed individuals, at the beginning of exposure; linear regression

cells produce a unique range of cytokines and chemokines needed for supporting survival and proliferation of B-cells in the germinal centers [9, 21, 36, 37]. Thus, $Tfh_{17}$ produce IL21 and IL17, which are involved in enhancing the interaction between T and B cells and are necessary for the formation of germinal centers [38].

Thus, in the exposed individuals, the late effects after chronic exposure to radiation include some changes in the T helper part of the immune system, which depend both on the radiation dose and on the age reached at the time of the study. However, the limited sample recruited for this study disallows unequivocal conclusions at this stage and necessitates further research.

## CONCLUSIONS

1. The long term effects of chronic exposure to radiation with the doses predominantly accumulated by the RBM (average dose — 567 ± 73 mGy), as registered in the Techa Riverside residents aged 67 through 84, do not include changes in the relative content of various T helper subpopulations in peripheral blood. 2. The relative amount of double positive $Tfh_{17}$ contained in the peripheral blood $T_{CM}$ population of the exposed individuals positively correlated with the degree of irradiation of thymus and peripheral lymphoid organs ($p$ = 0.02); however, there was no linear regression dependence registered. 3. The data obtained indirectly indicate intensification of involutional processes in the exposed individuals due to the number of $T_{Naive}$ decreasing with age and the number of $T_{EM}$ increasing therewith. In the exposed individuals group, the following late age-related dependences were revealed: decreasing relative amount of $T_{Naive}$ ($p$ = 0.009) and double positive $Tfh_{17}$ in the $T_{EM}$ subpopulation ($p$ = 0.04), and increasing relative content of $T_{EM}$ ($p$ = 0.04 ) in the peripheral blood. However, no similar dependencies were found in the control group.

**References**


1. Akleev AA, Blinova EA, Dolgushin II. TCR-mutacii v limfocitax periphericheskoj krovi i immunnyj status u lic, podvergshixsya xronicheskomu radiacionnomu vozdejstviyu, v otdalennye sroki. Rossijskij immunologicheskij zhurnal. 2019; 13 (22,1): 13–23. DOI: 10.31857/S102872210005016-2. Russian.
2. Nalbant A. IL17, IL21, and IL22 Cytokines of T Helper 17 Cells in Cancer. J Interferon Cytokine Res. 2019; 39 (1): 56–60. DOI: 10.1089/jir.2018.0057.
3. Akiyama M, Late effects of radiation on the human immune system: an overview of immune response among the atomic-bomb survivors. Int J Radiat Biol. 1995; 68: 497–508.
4. Heylmann D, Rödel F, Kindler T, Kaina B. Radiation sensitivity of human and murine peripheral blood lymphocytes, stem and progenitor cells. Biochimica et Biophysica Acta (BBA). Reviews on Cancer. 2014; 1846 (1): 121–29. DOI: 10.1016/j.bbcan.2014.04.009.
5. Rybkina VL, Bannikova MV, Adamova GV, Dörr H, Scherthan H, Azizova TV. Immunological markers of chronic occupational radiation exposure. Health Phys. 2018; 115 (1): 108–13. DOI:10.1097/HP.0000000000000855.
6. Akleev AV, redaktor. Posledstviya radioaktivnogo zagryazneniya reki Techi. Chelyabinsk: Kniga, 2016; 390 s. Russian.
7. Degteva MO, Napier BA, Tolstykh EI, Shishkina EA, Bougrov NG, Krestinina LYu, et al. Raspredelenie individual'nyx doz v kogorte lyudej, obluchennyx v rezul'tate radioaktivnogo zagryazneniya reki Techi. Medicinskaya radiologiya i radiacionnaya bezopasnost'. 2019; 64 (3): 46–53. DOI: 10.12737/article_5cf2364cb49523.98590475. Russian.
8. SanPin 2.6.1.2523-09 «Normy radiacionnoj bezopasnosti (NRB - 99/2009)». M., 2009; 225 s. Russian.
9. Zurochka AV, Khaidukov SV, Kudryavtsev IV, Chereshnev VA. Protochnaya citometriya v biomedicinskix issledovaniyax. Ekaterinburg: RIO UrO RAN, 2018; 720 s. Russian.
10. Kudryavtsev IV, Serebryakova MK, Totolyan AA. Znacheniya normy subpopulyacij T-xelperov razlichnogo urovnya differencirovki v periphericheskoj krovi. Klinicheskaya laboratornaya diagnostika. 2016; 61 (3): 179–84. DOI: 10.18821/0869-2084-2016-3-179-184. Russian.
11. Kudryavtsev IV, Borisov AG, Krobinets II, Savchenko AA, Serebryakova MK, Totolyan AA. Xemokinovye receptory na T-xelperax razlichnogo urovnya differencirovki: osnovnye subpopulyacii. Medicinskaya immunologiya. 2016; 18 (3): 239–50. DOI: 10.15789/1563-0625-2016-3-239-250. Russian.
12. Kudryavcev IV. T-kletki pamyati: osnovnye populyacii i stadii diferencirovki. Rossijskij immunologicheskij zhurnal. 2014; 8 (17): 947–64. Russian.
13. Dong C. Cytokine Regulation and Function in T Cells. Annu Rev Immunol. 2021; 39: 51–76. DOI: 10.1146/annurev-immunol-061020-053702.
14. Goronzy JJ, Lee WW, Weyand CM. Aging and T-cell diversity. Exp Gerontol. 2007; 42 (5): 400–6. DOI: 10.1016/j.exger.2006.11.016.
15. Lefebvre JS, Lorenzo EC, Masters AR, et al. Vaccine efficacy and T helper cell differentiation change with aging. Oncotarget. 2016; 7 (23): 33581–94. DOI: 10.18632/oncotarget.9254.
16. Haynes L, Lefebvre JS. Age-related Deficiencies in Antigen-Specific CD4 T cell Responses: Lessons from Mouse Models. Aging Dis. 2011; 2 (5): 374–81.
17. Huang J, Xu X, Yang J. miRNAs Alter T Helper 17 Cell Fate in the Pathogenesis of Autoimmune Diseases. Front Immunol. 2021; 12: 593473. DOI: 10.3389/fimmu.2021.593473.
18. Takeuchi Y, Hirota K, Sakaguchi S. Impaired T cell receptor signaling and development of T cell-mediated autoimmune arthritis. Immunol Rev. 2020; 294 (1): 164–76. DOI: 10.1111/imr.12841.
19. Shao F, Zheng P, Yu D, Zhou Z, Jia L. Follicular helper T cells in type 1 diabetes. FASEB J. 2020; 34 (1): 30–40. DOI: 10.1096/fj.201901637R.
20. Chang SH. T helper 17 (Th17) cells and interleukin-17 (IL17) in cancer. Arch Pharm Res. 2019; 42 (7): 549–59. DOI: 10.1007/s12272-019-01146-9.
21. Wu X, Fajardo-Despaigne JE, Zhang C, et al. Altered T Follicular Helper Cell Subsets and Function in Chronic Lymphocytic Leukemia. Front Oncol. 2021; 11: 674492. DOI: 10.3389/fonc.2021.674492.
22. Dahal LN. The dichotomy of T helper 17 cells in cancer. Nat Rev Immunol. 2017; 17 (9): 592. DOI: 10.1038/nri.2017.93.
23. Marshall EA, Ng KW, Kung SH, et al. Emerging roles of T helper 17 and regulatory T cells in lung cancer progression and metastasis. Mol Cancer. 2016; 15 (1): 67. DOI: 10.1186/s12943-016-0551-1.
24. Mazzarella G, Bianco A, Catena E, De Palma R, Abbate GF. Th1/Th2 lymphocyte polarization in asthma. Allergy. 2000; 55 (Suppl 61): 6–9. DOI: 10.1034/j.1398-9995.2000.00511.x.
25. Chen R, Wang YW, Fornace AJ Jr, Li HH. Impairment of the Intrinsic Capability of Th1 Polarization in Irradiated Mice: A Close Look at the Imbalanced Th1/Th2 Response after Irradiation. Radiat Res. 2016; 186 (6): 559–67. DOI: 10.1667/RR14401.1.
26. Zhang X-L, Komada Y, Chipeta J, Li Q-S, Inaba H, Azuma E, Yamamoto H, Sakurai M. Intracellular cytokine profile of T cells from children with acute lymphoblastic leukemia. Cancer Immunol Immunother. 2000; 49: 165–72.
27. Yatnda P, Mintz P, Grigoriadou K, Lemonnier F, Vilmer E, Langlade-Demoyen P. Analysis of T-cell defects in the specific immune response against acute lymphoblastic leukemia cells. Exp Hematol. 1999; 27: 1375–83.
28. De Totero D, Reato G, Mauro F, et al. IL-4 production and increased CD30 expression by a unique CD8 — T-cell subset in

B-cell chronic lymphocytic leukaemia. Br J Haematol. 1999; 104: 589–99.
29. Pan B, Zeng L, Cheng H, et al. Altered balance between Th1 and Th17 cells in circulation is an indicator for the severity of murine acute GVHD. Immunol Lett. 2012; 142 (1–2): 48–54. DOI: 10.1016/j.imlet.2011.12.005.
30. Bazzazi H, Aghaei M, Memarian A, Asgarian-Omran H., Behnampour N, Yazdani Y. Th1–Th17 ratio as a new insight in rheumatoid arthritis disease. Iran J Allergy Asthma Immunol. 2018; 17 (1): 68–77.
31. Kusunoki Y, Yamaoka M, Kasagi F, Hayashi T, MacPhee DG, Kyoizumi S. Long-lasting changes in the T-cell receptor V beta repertoires of CD4 memory T-cell populations in the peripheral blood of radiation-exposed people. Br J Haematol. 2003; 122: 975–84. DOI: 10.1046/j.1365-2141.2003.04520.x.
32. Yoshida K, Misumi M, Kubo Y, Yamaoka M, Kyoizumi S, Ohishi W, et al. Long-term effects of radiation exposure and metabolic status on telomere length of peripheral blood T cells in atomic bomb survivors. Radiat Res. 2016; 186: 367–76. DOI: 10.1667/RR14389.1.
33. Kiselev SM, Sokolnikov ME, Lyss LV, Ilyina NI. Immunological monitoring of the personnel at radiation hazardous facilities. Radiation Protection Dosimetry. 2016; 173 (1–3): 124–30. DOI: 10.1093/rpd/ncw346.
34. Rybkina VL, Azizova TV, Scherthan H, Meineke V, Doerr H, Adamova GV, et al. Expression of blood serum proteins and lymphocyte differentiation clusters after chronic occupational exposure to ionizing radiation. Radiat Environ Biophys. 2014; 53 (4): 659–70. DOI: 10.1007/s00411-014-0556-3.
35. Zhang H, Weyand CM, Goronzy JJ. Hallmarks of the aging T-cell system. FEBS J. 2021; 288 (24): 7123–142. DOI: 10.1111/febs.15770.
36. Bentebibel SE, Lopez S, Obermoser G, et al. Induction of ICOS+CXCR3+CXCR5+ TH cells correlates with antibody responses to influenza vaccination. Sci Transl Med. 2013; 5 (176): 176ra32. DOI: 10.1126/scitranslmed.3005191.
37. Brenna E, Davydov AN, Ladell K, et al. CD4+ T Follicular Helper Cells in Human Tonsils and Blood Are Clonally Convergent but Divergent from Non-Tfh CD4+ Cells. Cell Rep. 2020; 30 (1): 137–52. DOI: 10.1016/j.celrep.2019.12.016.
38. Olatunde AC, Hale JS, Lamb TJ. Cytokine-skewed Tfh cells: functional consequences for B cell help. Trends Immunol. 2021; 42 (6): 536–50. DOI: 10.1016/j.it.2021.04.006.

**Литература**

1. Аклеев А. А., Блинова Е. А, Долгушин И. И. TCR-мутации в лимфоцитах периферической крови и иммунный статус у лиц, подвергшихся хроническому радиационному воздействию, в отдаленные сроки. Российский иммунологический журнал. 2019; 13 (22,1): 13–23. DOI: 10.31857/S102872210005016-2.
2. Nalbant A. IL17, IL21, and IL22 Cytokines of T Helper 17 Cells in Cancer. J Interferon Cytokine Res. 2019; 39 (1): 56–60. DOI: 10.1089/jir.2018.0057.
3. Akiyama M, Late effects of radiation on the human immune system: an overview of immune response among the atomic-bomb survivors. Int J Radiat Biol. 1995; 68: 497–508.
4. Heylmann D, Rödel F, Kindler T, Kaina B. Radiation sensitivity of human and murine peripheral blood lymphocytes, stem and progenitor cells. Biochimica et Biophysica Acta (BBA). Reviews on Cancer. 2014; 1846 (1): 121–29. DOI: 10.1016/j.bbcan.2014.04.009.
5. Rybkina VL, Bannikova MV, Adamova GV, Dörr H, Scherthan H, Azizova TV. Immunological markers of chronic occupational radiation exposure. Health Phys. 2018; 115 (1): 108–13. DOI:10.1097/HP.0000000000000855.
6. Аклеев А. В., редактор. Последствия радиоактивного загрязнения реки Течи. Челябинск: Книга, 2016; 390 с.
7. Дегтева М. О., Напье Б. А., Толстых Е. И., Шишкина Е. А., Бугров Н. Г., Крестинина Л. Ю., Аклеев А. В. Распределение индивидуальных доз в когорте людей, облученных в результате радиоактивного загрязнения реки Течи. Медицинская радиология и радиационная безопасность. 2019; 64 (3): 46–53. DOI: 10.12737/article_5cf2364cb49523.98590475.
8. СанПин 2.6.1.2523-09 «Нормы радиационной безопасности (НРБ - 99/2009)». М., 2009; 225 с.
9. Зурочка А. В., Хайдуков С. В., Кудрявцев И. В., Черешнев В. А. Проточная цитометрия в биомедицинских исследованиях. Екатеринбург: РИО УрО РАН, 2018; 720 с.
10. Кудрявцев И. В., Серебрякова М. К., Тотолян А. А. Значения нормы субпопуляций Т-хелперов различного уровня дифференцировки в периферической крови. Клиническая лабораторная диагностика. 2016; 61 (3): 179–84. DOI: 10.18821/0869-2084-2016-3-179-184.
11. Кудрявцев И. В., Борисов А. Г., Кробинец И. И., Савченко А. А., Серебрякова М. К., Тотолян А. А. Хемокиновые рецепторы на Т-хелперах различного уровня дифференцировки: основные субпопуляции. Медицинская иммунология. 2016; 18 (3): 239–50. DOI: 10.15789/1563-0625-2016-3-239-250.
12. Кудрявцев И. В. Т-клетки памяти: основные популяции и стадии диференцировки. Российский иммунологический журнал. 2014; 8 (17): 947–64.
13. Dong C. Cytokine Regulation and Function in T Cells. Annu Rev Immunol. 2021; 39: 51–76. DOI: 10.1146/annurev-immunol-061020-053702.
14. Goronzy JJ, Lee WW, Weyand CM. Aging and T-cell diversity. Exp Gerontol. 2007; 42 (5): 400–6. DOI: 10.1016/j.exger.2006.11.016.
15. Lefebvre JS, Lorenzo EC, Masters AR, et al. Vaccine efficacy and T helper cell differentiation change with aging. Oncotarget. 2016; 7 (23): 33581–94. DOI: 10.18632/oncotarget.9254.
16. Haynes L, Lefebvre JS. Age-related Deficiencies in Antigen-Specific CD4 T cell Responses: Lessons from Mouse Models. Aging Dis. 2011; 2 (5): 374–81.
17. Huang J, Xu X, Yang J. miRNAs Alter T Helper 17 Cell Fate in the Pathogenesis of Autoimmune Diseases. Front Immunol. 2021; 12: 593473. DOI: 10.3389/fimmu.2021.593473.
18. Takeuchi Y, Hirota K, Sakaguchi S. Impaired T cell receptor signaling and development of T cell-mediated autoimmune arthritis. Immunol Rev. 2020; 294 (1): 164–76. DOI: 10.1111/imr.12841.
19. Shao F, Zheng P, Yu D, Zhou Z, Jia L. Follicular helper T cells in type 1 diabetes. FASEB J. 2020; 34 (1): 30–40. DOI: 10.1096/fj.201901637R.
20. Chang SH. T helper 17 (Th17) cells and interleukin-17 (IL17) in cancer. Arch Pharm Res. 2019; 42 (7): 549–59. DOI: 10.1007/s12272-019-01146-9.
21. Wu X, Fajardo-Despaigne JE, Zhang C, et al. Altered T Follicular Helper Cell Subsets and Function in Chronic Lymphocytic Leukemia. Front Oncol. 2021; 11: 674492. DOI: 10.3389/fonc.2021.674492.
22. Dahal LN. The dichotomy of T helper 17 cells in cancer. Nat Rev Immunol. 2017; 17 (9): 592. DOI: 10.1038/nri.2017.93.
23. Marshall EA, Ng KW, Kung SH, et al. Emerging roles of T helper 17 and regulatory T cells in lung cancer progression and metastasis. Mol Cancer. 2016; 15 (1): 67. DOI: 10.1186/s12943-016-0551-1.
24. Mazzarella G, Bianco A, Catena E, De Palma R, Abbate GF. Th1/Th2 lymphocyte polarization in asthma. Allergy. 2000; 55 (Suppl 61): 6–9. DOI: 10.1034/j.1398-9995.2000.00511.x.
25. Chen R, Wang YW, Fornace AJ Jr, Li HH. Impairment of the Intrinsic Capability of Th1 Polarization in Irradiated Mice: A Close Look at the Imbalanced Th1/Th2 Response after Irradiation. Radiat Res. 2016; 186 (6): 559–67. DOI: 10.1667/RR14401.1.
26. Zhang X-L, Komada Y, Chipeta J, Li Q-S, Inaba H, Azuma E, Yamamoto H, Sakurai M. Intracellular cytokine profile of T cells from children with acute lymphoblastic leukemia. Cancer Immunol Immunother. 2000; 49: 165–72.
27. Yatnda P, Mintz P, Grigoriadou K, Lemonnier F, Vilmer E, Langlade-Demoyen P. Analysis of T-cell defects in the specific immune response against acute lymphoblastic leukemia cells. Exp Hematol. 1999; 27: 1375–83.

28. De Totero D, Reato G, Mauro F, et al. IL-4 production and increased CD30 expression by a unique CD8 — T-cell subset in B-cell chronic lymphocytic leukaemia. Br J Haematol. 1999; 104: 589–99.
29. Pan B, Zeng L, Cheng H, et al. Altered balance between Th1 and Th17 cells in circulation is an indicator for the severity of murine acute GVHD. Immunol Lett. 2012; 142 (1–2): 48–54. DOI: 10.1016/j.imlet.2011.12.005.
30. Bazzazi H, Aghaei M, Memarian A, Asgarian-Omran H., Behnampour N, Yazdani Y. Th1–Th17 ratio as a new insight in rheumatoid arthritis disease. Iran J Allergy Asthma Immunol. 2018; 17 (1): 68–77.
31. Kusunoki Y, Yamaoka M, Kasagi F, Hayashi T, MacPhee DG, Kyoizumi S. Long-lasting changes in the T-cell receptor V beta repertoires of CD4 memory T-cell populations in the peripheral blood of radiation-exposed people. Br J Haematol. 2003; 122: 975–84. DOI: 10.1046/j.1365-2141.2003.04520.x.
32. Yoshida K, Misumi M, Kubo Y, Yamaoka M, Kyoizumi S, Ohishi W, et al. Long-term effects of radiation exposure and metabolic status on telomere length of peripheral blood T cells in atomic bomb survivors. Radiat Res. 2016; 186: 367–76. DOI: 10.1667/RR14389.1.
33. Kiselev SM, Sokolnikov ME, Lyss LV, Ilyina NI. Immunological monitoring of the personnel at radiation hazardous facilities. Radiation Protection Dosimetry. 2016; 173 (1–3): 124–30. DOI: 10.1093/rpd/ncw346.
34. Rybkina VL, Azizova TV, Scherthan H, Meineke V, Doerr H, Adamova GV, et al. Expression of blood serum proteins and lymphocyte differentiation clusters after chronic occupational exposure to ionizing radiation. Radiat Environ Biophys. 2014; 53 (4): 659–70. DOI: 10.1007/s00411-014-0556-3.
35. Zhang H, Weyand CM, Goronzy JJ. Hallmarks of the aging T-cell system. FEBS J. 2021; 288 (24): 7123–142. DOI: 10.1111/febs.15770.
36. Bentebibel SE, Lopez S, Obermoser G, et al. Induction of ICOS+CXCR3+CXCR5+ TH cells correlates with antibody responses to influenza vaccination. Sci Transl Med. 2013; 5 (176): 176ra32. DOI: 10.1126/scitranslmed.3005191.
37. Brenna E, Davydov AN, Ladell K, et al. CD4+ T Follicular Helper Cells in Human Tonsils and Blood Are Clonally Convergent but Divergent from Non-Tfh CD4+ Cells. Cell Rep. 2020; 30 (1): 137–52. DOI: 10.1016/j.celrep.2019.12.016.
38. Olatunde AC, Hale JS, Lamb TJ. Cytokine-skewed Tfh cells: functional consequences for B cell help. Trends Immunol. 2021; 42 (6): 536–50. DOI: 10.1016/j.it.2021.04.006.