Assessment of Some Immune Parameters in Occupationally Exposed Nuclear Power Plant Workers: Flow Cytometry Measurements of T Lymphocyte Subpopulations and Immunoglobulin Determination

Ilona Mihaylova Gyuleva¹, Kalina Ivanova Penkova², Ivanka Tankova Rupova¹, Delyana Yonkova Panova¹, and Jana Nikolaeva Djounova¹

Abstract
A 10-year survey of immune status of nuclear power plant (NPP) workers was assessed by cellular and humoral immune parameters. The cumulative doses of NPP workers were in the range of 0.06 to 766.36 mSv. The results did not show significant deviations in the studied parameters of cellular and humoral immunity, but a tendency of elevated values in CD3⁺4⁺ helper inducers cells, especially its CD4⁺62L⁺ subpopulation, regulatory CD4⁺25⁺ cells, CD8⁺28⁺ cytotoxic subpopulation, and immunoglobulin M, was established. The observed trend of the above-mentioned parameters could be interpreted by assumption that while the adaptation processes are dominated with low prevalence of T-helper (Th) 1 immune response to cumulative doses less than 100 mSv, a switch to Th-2 response occurred at doses above 100 mSv. The impact of a number of other confounding factors on the immune system does not allow definitive conclusions about the direct radiation-induced changes in immune parameters.

Keywords
immunology, ionizing radiation, low-dose effects, lymphocytes, immunoglobulin

Introduction
A key issue in radiobiology is the health risk assessment. To solve this problem, special attention is paid to the health status of workers chronically exposed to low-dose radiation in nuclear industry. The radiological hazard associated with nuclear generation are notably different from the occupational hazard associated with other methods for power generation. Epidemiological studies¹⁴ of nuclear power plant (NPP) contingents are controversial because of other factors such as living conditions, harmful habits, and improved radiation protection measures in the recent years. Effects of chronic low-dose radiation on changes in immunological parameters and state of subclinical inflammation require careful examination of the immune status of occupationally exposed persons. Some authors found that doses in the range of 10 to 100 mGy lead to prevalence of T1 helper subpopulation (Th1), while doses above 200 mGy switch the prevalence of T2 immune response which determines an increased risk of neoprocesses, infectious diseases, allergies, and autoimmune diseases.⁵⁻⁸ At the same time, experimental data established that low doses stimulate antitumor immunity manifested by increased activity of T, natural killer (NK) cells, and B cells and higher production of interleukin (IL) 2 (IL-2), IL-12, interferon γ (IFN-γ) cytokines, and induced T helper 1 immune response.⁹⁻¹¹

Assessment of the main lymphocyte populations of the NPP workers done by us for the last 10 years didn’t show significant

¹ National Centre Radiobiology and Radiation Protection, Sofia, Bulgaria
² Aleksandrovska Hospital, Sofia, Bulgaria

Corresponding Author:
Ilona Mihaylova Gyuleva, National Centre Radiobiology and Radiation Protection, Georgy Sofiiski str 3, Sofia, 1606, Bulgaria.
Email: iguleva@yahoo.com
differences in the main lymphocyte populations—B and T lymphocytes and NK cells.12 Although the average values of studied populations were within the reference limits, some trends were obtained at the statistical processing of the results. The more pronounced deviations found in T lymphocytes, as well as reports of radiation-induced imbalance between TH1 and TH2 immune responses,5,10,6-8 suggested the need to include additional indicators aimed at the expansion and refinement of the studied immune parameters.

Materials and Methods

The studied participants consisted of 2 groups, one of 438 employees (421 males and 17 females) working in NPP Kozloduy, Bulgaria, and the other of 65 control persons (49 males and 16 females) at similar age, sex, and length of service without any work-related exposure to ionizing radiation. The study was conducted under contracts between the National Centre of Radiobiology and Radiation Protection of the Ministry of Health of Bulgaria and the NPP Kozloduy aiming to carry out health monitoring and study potential changes in the immunological status of NPP workers due to low-dose occupational exposure. Informed consent was obtained from all participants.

The group of individuals occupationally exposed to external γ radiation was selected from the service personnel of units 5 and 6 of NPP Kozloduy. The radiation doses were determined by NPP individual exposure monitoring program.

For the purposes of our research, the workers were divided into groups according to the cumulative dose received as follows: internal control; up to dose of 20 mSv; up to dose of 100 mSv; up to dose of 200 mSv; and above dose of 200 mSv (Table 1).

After filling in a questionnaire, all participants were subjected to medical examinations and underwent a basic hematological assay to evaluate their health status. Although no deviations in the basic laboratory tests and current infections were found in any of the respondents, some of them were diagnosed with cardiovascular diseases (hypertension and ischemic heart disease), obesity, undiagnosed chronic hepatitis 10 years ago, and other chronic diseases whose distribution among the groups is illustrated in Table 2.

### Table 1. NPP Personnel: Groups According to Received Doses, Average Age and Length of Employment.

| Personnel | Group | Dose, mSv | n          | Average Age, Years± | Lenght of Service, Years± | Received Dose, mSv± |
|-----------|-------|-----------|------------|---------------------|--------------------------|---------------------|
| Control   | 0     | 65 (49 males and 16 females) | 46 ± 8     | 19 ± 8              | 0                        |
| 1         | 0.1-20| 103 (93 males and 10 females) | 43 ± 7     | 16 ± 8              | 9.53 ± 5.8               |
| 2         | 20.1-100| 117 (112 males and 5 females) | 42 ± 8     | 16 ± 8              | 49.42 ± 21.7            |
| 3         | 100.1-200 | 66 (64 males and 2 females) | 45 ± 7     | 18 ± 6              | 141.95 ± 30.4           |
| 4         | >200   | 153 (males) | 45 ± 6     | 20 ± 6              | 354.48 ± 120.3          |

Abbreviation: NPP, nuclear power plant.

* Data indicate the standard deviation (±SD).

### Table 2. Health Status of NPP Workers.

| Condition | Healthy | With disease | Control, % | 0.1-20 mSv, % | 20-100 mSv, % | 100-200 mSv, % | >200, mSv, % |
|-----------|---------|--------------|------------|--------------|-------------|--------------|-------------|
| Mobius hypertonicus | 81.8 | 18.2 | Healthy | 66.7 | 33.3 | 64.5 | 35.5 | 68.4 | 31.6 | 62.1 |
| Cardio vascular diseases | 78.8 | 21.2 | Healthy | 66.7 | 33.3 | 64.5 | 35.5 | 63.2 | 36.8 | 37.9 |
| Obesities | Healthy | 84.8 | 15.2 | 85.2 | 14.8 | 74.2 | 25.8 | 42.1 | 57.9 | 93.1 |
| Diabetes mellitus | 97.0 | 3.0 | Healthy | 926 | 7.4 | 96.8 | 3.2 | 100.0 | 3.2 | 96.6 |
| Hepatitis (inactive, not chronic) | Healthy | 97.0 | 3.0 | 88.9 | 11.1 | 90.3 | 9.7 | 89.5 | 10.5 | 93.1 |
| Vaccinations and virus diseases in last month | Healthy | 84.8 | 15.2 | 85.2 | 14.8 | 93.5 | 6.5 | 89.5 | 10.5 | 89.7 |
| Gastrointestinal diseases | Healthy | 89.2 | 10.8 | 93.6 | 6.4 | 93.4 | 6.6 | 94.9 | 5.1 | 94.1 |
| Pulmonary diseases | Healthy | 100.0 | 10.8 | 96.8 | 3.2 | 97.8 | 2.2 | 96.6 | 3.4 | 97.4 |

* Data are presented as percent of all individuals in each group.
Four milliliters of blood was collected from each participant by venipuncture into Vacuette EDTA tubes (Greiner Bio-One GmbH, Kremsmunster, Austria). Total white blood cells (WBC) and lymphocytes were counted using an automatic hematology analyzer ABX Pentra 60 C+ (HORIBA GROUP ABX Diagnostics, Montpellier, France) operated in cell blood count + 5 population differential count (CBC + 5 DIFF) modes. Blood smears were prepared to observe the WBC morphology. The blood samples were processed, and whole blood was stained with fluorescein isothiocyanate (FITC)- and phycoerythrin (PE)-conjugated monoclonal antibodies within 6 hours after withdrawal. Lymphocyte subsets were examined as percentages of the lymphocyte count and identified by 2-color flow cytometry in whole blood following erythrocyte lysis according to the method of Jackson as described in Becton Dickinson Monoclonal Antibodies Source Book. Approximately $10^4$ events per sample were analyzed with FACS Calibur flow cytometer (Becton Dickenson Biosciences, San Jose, California). The lymphocyte fraction was gated by forward and right-angle light scatter. Simulset software automatically collected a sufficient number of events to obtain a minimum of 2000 lymphocytes within lymphocyte gate. In addition to the main lymphocyte populations CD3+, CD19+, CD4+, and CD8+, tested in a previous study, in the present investigation CD4+25+, CD4+62L−, CD4+62L+, CD8+28+, CD8+38+ were also analyzed. Simulset IMK-Lymphocyte kit, BD Simulset CD4 FITC/CD62L PE, BD Simulset CD8 FITC/CD28 PE, BD Simulset CD8 FITC/CD38 PE, CD4 FITC, and CD25 PE (Becton Dickinson Biosciences, San Jose, California, USA) were used.

Humoral immunity was studied by the standard method of radial immunodiffusion, and serum levels of immunoglobulin (Ig) G, IgA, and IgM were determined using immunoplates (ARCO Inc. Bulgaria, distributer of LTA, Italy).

The reference values for the Bulgarian population defined by the Central Laboratory of Clinical Immunology, University Hospital “Alexandrovska” (Sofia, Bulgaria); 5th percentile to 95th percentile range.

### Table 3. Reference Values* for Lymphocyte Subsets and Immunoglobulin.

| Lymphocyte Subsets as Percentage of Lymphocyte Count | Percentage Values | Absolute Values, cell/μL |
|-----------------------------------------------------|-------------------|--------------------------|
| CD4⁺CD62L⁻ | 6%-20% | 120-460 |
| CD4⁺CD62L⁺ | 22%-60% | 620-1050 |
| CD4⁺CD25⁺ | 5%-14% | 20-240 |
| CD8⁺CD28⁺ | 3%-11% | 210-438 |
| CD8⁺CD38⁺ | 13%-26% | 230-550 |

| Immunoglobulins | Values, g/L |
|-----------------|-------------|
| IgG             | 0.8-1.8     |
| IgA             | 0.9-4.50    |
| IgM             | 0.7-2.80    |
| IgG             | 0.8-1.8     |

* Reference values for the Bulgarian population provided by the Central Laboratory of Clinical Immunology, University Hospital “Alexandrovska” (Sofia, Bulgaria); 5th percentile to 95th percentile range.

### Statistical Methods

The following statistical methods were applied to process the results.

**Variation analysis of quantitative variables:** Mean, standard deviation, standard error of the mean, and 95% confidence interval of the mean were calculated.

Statistical analysis was performed using parametric and nonparametric methods as follows:

- **Parametric methods:** 1-way analysis of variance (ANOVA) to check the equality of more than 2 mean values in a normal distribution.
- **Nonparametric methods:** Kolmogorov-Smirnov and Shapiro-Wilk tests to check the normality of distribution of quantitative variables; Mann-Whitney $U$ test for comparison of averages in 2 groups of 1 quantitative variable when the distribution is not normal; and Kruskal-Wallis test for comparison of averages in more than 2 groups of quantitative variables when the distribution is not normal.
- **Frequency analysis:** The frequencies of observations occurring in certain ranges of values.
- **Correlation analysis:** Coefficient of linear correlation, parametric (Pearson), and nonparametric (Spearman) analysis.

SPSS version 11.0.1 for Windows was used for data processing.

### Results and Discussion

In our previous study on the same contingent of NPP workers, no significant differences were found between the exposed groups and the control regarding the main lymphocyte populations: B and T lymphocytes and NK cells with phenotypes CD3⁺16⁺,56⁺,12⁺.

Although the average values were within the reference limits, a trend of decreasing total CD3⁺ T lymphocyte was obtained mostly on the account of significant reduction in CD4⁺ T helper cells with increasing cumulative doses of up to 200 mSv. The heterogeneity of CD3⁺4⁺ population and its subpopulations CD4⁺62L⁻ and CD4⁺62L⁺ T cells, as well as activated CD4⁺25⁺ T lymphocytes, were investigated for its more precise determination (Figure 1).

Lower average percentage values were observed for CD3⁺4⁺ lymphocytes in groups with cumulative doses below 200 mSv, statistical confidence was established in the first and third groups, and at doses above 200 mSv there was a tendency of increasing their number (Mann-Whitney $U$ test).
subpopulation, mediating helper subpopulation remained at T lymphocytes in the prevention of autoimmune, + subpopulation inducing suppression of T cells. At doses P cells (Table 6). This could contribute to stress changes them reciprocally—reduced CD62L—and induced changes in manifestation of CD62L adhesion molecules, suggesting that acute stress leads to increased CD62L— against CD62L+ on lymphocytes, while chronic stress changes them reciprocally—reduced CD62L—and increased CD62L+ lymphocytes. It cannot conclusively be said whether the observed increase in average CD4+62L+ cell subpopulation at cumulative doses above 100 mSv is determined by the chronic stress, including radiation impact or probably both mechanisms are involved.

**CD4+CD62L−**

The data for CD4+62L− subpopulation, mediating helper function in B lymphocyte differentiation, showed a lack of statistically significant differences between the groups and the control (ANOVA; Table 4).

More individuals with elevated above-normal values for CD4+62L− subpopulation were established in the first and fourth groups (Table 5), as the majority of them were smokers (P = .044; Table 6). No correlation of this parameter was found with the dose received, age, and length of service for the observed personnel.

**CD4+CD62L+**

Statistically significant reduction in the mean proportional values of CD4+62L+ subpopulation inducing suppression of B lymphocyte differentiation was found in the first and second groups. Comparison within groups showed statistically significant differences between the mean values in the first and fourth groups (P = .003) and second and fourth groups (P = .021). Upward trend in the average number of this parameter was established with increase in cumulative dose up to 200 mSv, and thereafter it remained at the same level (Table 4).

Frequency analysis showed the highest percentage of persons with values below the reference in the first and second groups, 21.8% and 22.8%, respectively (Table 5). The consumption of alcohol and cigarettes was least significant (P = .017) favoring mainly deviations in this subpopulation (Table 6).

There was a significant weak positive correlation of the relative values of CD4+62L+ lymphocytes with the length of service of respondents (r = .118 at P = .017). The results indicated that with increasing cumulative dose up to 200 mSv, the growth of CD4+62L+ subpopulation was significant and repeated the tendency observed for CD3+4+ cells. At doses above 200 mSv, an elevation in CD4+CD62L cells was observed, but CD4+CD62L+ subpopulation remained at almost the same number. Some authors have reported that the memory CD45RO+CD4+ cells could be divided into 2 subgroups based on expression of L-selectin receptor (CD62L). It was found that the memory CD4+CD62L+ lymphocytes predominantly produce IL-4 and IL-5 cytokine profile specific for Th2 immune response, while the memory L-selectin negative CD4+ T cells produce IL-17 and IL-22 cytokines leading to Th1 response. Whether the reduced values of CD4+62L+ lymphocytes in participants with cumulative doses below 100 mSv express prevalence of Th1 response at low doses still remains an assumption due to incomplete research (haven’t distinguished memory and naive cells) but is an important issue for future studies.

The analysis of other adverse factors such as smoking showed an increase in the average CD4+62L− values and a reduction in CD4+62L+ cells (Table 6). This could contribute to the observed tendency assuming that the predominant respondents were smokers. Other studies showed stress-induced changes in manifestation of CD62L adhesion molecules, suggesting that acute stress leads to increased CD62L− against CD62L+ on lymphocytes, while chronic stress changes them reciprocally—reduced CD62L—and increased CD62L+ lymphocytes. It cannot conclusively be said whether the observed increase in average CD4+62L+ cell subpopulation at cumulative doses above 100 mSv is determined by the chronic stress, including radiation impact or probably both mechanisms are involved.

**CD4+25+ T Lymphocyte**

Recently, a number of publications deal with the role of CD4+25+ T lymphocytes in the prevention of autoimmune, infectious, and inflammatory diseases. Since such phenotypic characteristics have both activated helper inducer and regulatory CD4+25+ lymphocytes (which are distinguished by the degree of expression of CD127 molecule), it is also necessary to determine greater accuracy for this receptor in further studies. It is known that ionizing radiation producing functional alteration of the immune system and breaking self-tolerance could cause autoimmune diseases. Sakaguchi et al experimentally demonstrated that elimination of CD4+CD25+ T regulatory cells led to development of various organ-specific autoimmune diseases in mice.

By applying the Mann-Whitney U test, statistically significant decrease in CD4+25+ was found in the first group compared to control, followed by a tendency of gradual increase in the average percentage values to those of the control up to the fourth group (Figure 2). The comparison between the groups showed statistically significant difference in mean results between first and third groups (P = .006) and for the proportion of CD4+CD25+ population to all CD3+4+ T lymphocytes between first and fourth groups (P < .000; Table 4). The frequency analysis indicated the highest percentage of persons with elevated values for this parameter in the control group.
| Parameters                          | Groups                                      | Persons, N | X ± SD, % | X ± SD, cells/μL | X ± SD,subp%/popul% |
|------------------------------------|---------------------------------------------|------------|-----------|------------------|--------------------|
| **CD4+CD62L− T lymphocytes**       | Control                                     | 62         | 11.89 ± 4.2 | 269.74 ± 139.3   |                    |
|                                   | First group: doses 0.1-20 mSv               | 92         | 12.56 ± 6.1 | 290.03 ± 142.7   | P ≥ .05            |
|                                   | Second group: doses 20.1-100 mSv            | 76         | 12.05 ± 5.1 | 266.93 ± 131.3   | P ≥ .05            |
|                                   | Third group: doses 100.1-200 mSv            | 56         | 11.04 ± 5.1 | 265.96 ± 126.4   | P ≥ .05            |
|                                   | Fourth group: doses above 200 mSv           | 123        | 12.09 ± 4.4 | 292.30 ± 135.5   | P ≥ .05            |
| **CD4+CD62L+ T lymphocytes**       | Control                                     | 62         | 32.24 ± 6.4 | 712.03 ± 249.6   |                    |
|                                   | First group: doses 0.1-20 mSv               | 92         | 27.77 ± 8.3 | 645.86 ± 249.5   | P < .000           |
|                                   | Second group: doses 20.1-100 mSv            | 76         | 28.76 ± 6.7 | 648.88 ± 256.7   | P < .002           |
|                                   | Third group: doses 100.1-200 mSv            | 56         | 29.16 ± 9.6 | 735.89 ± 315.4   | P = .065           |
|                                   | Fourth group: doses above 200 mSv           | 123        | 31.24 ± 8.5 | 732.56 ± 269.9   | P ≥ .05            |
| **CD4+25+ T lymphocytes**          | Control                                     | 64         | 12.47 ± 6.64| 276.9 ± 162.2    | 27.88 ± 13.7       |
|                                   | First group: doses 0.1-20 mSv               | 94         | 9.38 ± 5.55 | 217.4 ± 140.8    | P = .002           |
|                                   | Second group: doses 20.1-100 mSv            | 81         | 10.83 ± 6.44| 234.9 ± 170.0    | P = .009           |
|                                   | Third group: doses 100.1-200 mSv            | 59         | 11.78 ± 5.73| 293.9 ± 176.1    | P ≥ .05            |
|                                   | Fourth group: doses above 200 mSv           | 123        | 12.06 ± 6.02| 294.7 ± 175.0    | P ≥ .05            |

(continued)
| Parameters                        | Groups                                      | Persons, N | $X \pm SD, \%$ | $X \pm SD, \text{cells/μL}$ | $X \pm SD, \text{subp%/popul%}$ | CD8$^{+}$28$^{\%}$/ CD8$^{\%}$ |
|----------------------------------|---------------------------------------------|------------|----------------|-----------------------------|-------------------------------|---------------------------------|
| Cytotoxic T lymphocytes (CD8$^{+}$28$^{\%}$) | Control                                    | 61         | 14.85 ± 3.5   | 343.44 ± 143.0             | 58.80 ± 12.6                 |                                 |
|                                  | First group: doses 0.1-20 mSv               | 94         | 15.88 ± 4.9   | 366.22 ± 135.7             | 55.67 ± 18.1                 |                                 |
|                                  | $P \geq .05$                               |            |               | $P \geq .05$               | $P \geq .05$                |                                 |
|                                  | Second group: doses 20.1-100 mSv            | 74         | 15.20 ± 4.8   | 343.62 ± 157.9             | 56.12 ± 16.4                 |                                 |
|                                  | $P \geq .05$                               |            |               | $P \geq .05$               | $P \geq .05$                |                                 |
|                                  | Third group: doses 100.1-200 mSv            | 55         | 14.07 ± 4.0   | 351.42 ± 132.4             | 57.61 ± 20.5                 |                                 |
|                                  | $P \geq .05$                               |            |               | $P \geq .05$               | $P \geq .05$                |                                 |
|                                  | Fourth group: doses above 200 mSv           | 123        | 13.48 ± 3.7   | 325.47 ± 136.1             | 51.40 ± 17.3                 |                                 |
|                                  | $P < .009$                                 |            |               | $P \geq .05$               |                              |                                 |
| Activated CD3$^{+}$8$^{\%}$ T lymphocytes (CD8$^{+}$38$^{\%}$) | Control                                    | 44         | 14.86 ± 5.8   | 324.05 ± 160.2             |                              |                                 |
|                                  | First group: doses 0.1-20 mSv               | 58         | 16.91 ± 5.7   | 388.53 ± 157.4             |                              |                                 |
|                                  | $P = .054$                                 |            |               | $P \geq .05$               |                              |                                 |
|                                  | Second group: doses 20.1-100 mSv            | 46         | 16.85 ± 7.3   | 368.11 ± 174.4             |                              |                                 |
|                                  | $P \geq .05$                               |            |               | $P \geq .05$               |                              |                                 |
|                                  | Third group: doses 100.1-200 mSv            | 41         | 15.61 ± 8.8   | 368.07 ± 187.4             |                              |                                 |
|                                  | $P \geq .05$                               |            |               | $P \geq .05$               |                              |                                 |
|                                  | Fourth group: doses above 200 mSv           | 84         | 13.79 ± 5.6   | 339.59 ± 177.3             |                              |                                 |
|                                  | $P \geq .05$                               |            |               | $P \geq .05$               |                              |                                 |

Note: Boldface P values show significance of results.
Table 5. Frequency Analysis of Studied Immune Parameters in NPP Workers given as a Percentage of the Number of Participants Who had Cell Counts Under, Within, or Above Reference Range.

| Parameters | Groups                  | Under Reference Range | Reference Range | Above Reference Range |
|------------|-------------------------|-----------------------|-----------------|-----------------------|
| CD4+62L−   | Control                 | 6.3%                  | 88.9%           | 4.8%                  |
|            | First group             | 4.3%                  | 83.7%           | 12%                   |
|            | Second group            | 9.2%                  | 82.9%           | 7.9%                  |
|            | Third group             | 1.8%                  | 92.9%           | 5.4%                  |
|            | Fourth group            | 4.9%                  | 84.6%           | 10.6%                 |
| CD4+62L+   | Control                 | 42.9%                 | 46%             | 11.1%                 |
|            | First group             | 50%                   | 46.7%           | 3.3%                  |
|            | Second group            | 52.6%                 | 39.5%           | 7.9%                  |
|            | Third group             | 41.1%                 | 42.9%           | 16.1%                 |
|            | Fourth group            | 38.2%                 | 52%             | 9.8%                  |
| CD4+25+    | Control                 | 7.7%                  | 58%             | 33.8%                 |
|            | First group             | 18.1%                 | 63.8%           | 18.1%                 |
|            | Second group            | 13.6%                 | 61.7%           | 24.7%                 |
|            | Third group             | 8.5%                  | 62.7%           | 28.8%                 |
|            | Fourth group            | 9.8%                  | 62.6%           | 27.6%                 |
| CD8+28+    | Control                 | 22.6%                 | 74.2%           | 3.2%                  |
|            | First group             | 26.6%                 | 60.6%           | 12.8%                 |
|            | Second group            | 33.8%                 | 55.4%           | 10.8%                 |
|            | Third group             | 30.9%                 | 65.5%           | 3.6%                  |
|            | Fourth group            | 44.7%                 | 51.2%           | 4.1%                  |
| IgG        | Control                 | 13.6%                 | 73.8%           | 12.3%                 |
|            | First group             | 7.8%                  | 87.4%           | 4.9%                  |
|            | Second group            | 10.3%                 | 80.2%           | 9.5%                  |
|            | Third group             | 4.6%                  | 78.5%           | 16.9%                 |
|            | Fourth group            | 9.2%                  | 82.4%           | 8.5%                  |
| IgA        | Control                 | 4.6%                  | 86.2%           | 9.2%                  |
|            | First group             | 7.8%                  | 82.5%           | 9.7%                  |
|            | Second group            | 1.7%                  | 91.3%           | 7.0%                  |
|            | Third group             | 1.5%                  | 90.8%           | 7.7%                  |
|            | Fourth group            | 2.6%                  | 91.5%           | 5.9%                  |
| IgM        | Control                 | 4.6%                  | 87.7%           | 7.7%                  |
|            | First group             | 0%                    | 86.4%           | 13.6%                 |
|            | Second group            | 0.9%                  | 89.7%           | 9.5%                  |
|            | Third group             | 1.5%                  | 87.7%           | 9.8%                  |
|            | Fourth group            | 4.6%                  | 83.7%           | 11.8%                 |

Abbreviations: Ig, immunoglobulin; NPP, nuclear power plant.
found in the fourth group compared to control \((P < .009)\) as well as in intergroup analysis (Mann-Whitney \(U\) test) between fourth and first \((P < .000)\), fourth and second \((P = .018)\) groups, respectively (Table 4).

A significant weak negative correlation of the values with the received dose \((r = .203 \text{ at } P = .000)\) and the age \((r = .144 \text{ at } P = .004)\) was found. A tendency of reducing the relative values of cytotoxic CD8\(^+\)28\(^+\) T lymphocytes to total suppressor-cytotoxic T lymphocytes with increasing cumulative dose was observed which was confirmed by established negative correlation with dose \((r = .143 \text{ at } P = .004)\).

More individuals with elevated parameters above the reference values were in the first (12.8\%) and second groups (10.8\%; Table 5).

In individuals consuming alcohol, the percentage of deviations for studied parameter was significantly higher \((P = .019;\) Table 6). The observed increase in CD8\(^+\)28\(^+\) T lymphocytes in the first 2 groups which are dominated by younger people who declared higher alcohol consumption could be in confirm of the impact of alcohol on the studied immune parameter. A marked downward trend was observed for cytotoxic CD8\(^+\)28\(^+\) T subpopulation of CD3\(^+\)8\(^+\) lymphocytes and upward tendency for their suppressor CD828\(^-\) T lymphocytes with increase in cumulative dose. These results are supported by a study of Lui SZ et al\(^{25}\) who considered that low doses could increase expression of CD28 and lead to immune stimulation while high doses lead to CTLA-4 expression and immunosuppression.

The prevalence of suppressor T lymphocytes in groups with higher cumulative dose could be associated with compensatory activation of regulatory suppressor mechanisms in response of the polarization to humoral-type immune response with increasing in dose.

**Activated T lymphocytes (CD8\(^+\)38\(^+\))**

The results obtained for activated CD8\(^+\)38\(^+\) subpopulation of T lymphocytes analyzed by Kruskal-Wallis test did not show statistically significant differences in the average values of

| Parameters | Under Ref. Range | Reference Range | Above Ref. Range |
|------------|------------------|-----------------|------------------|
|            | Nonsmokers | Smokers | Nonsmokers | Smokers | Nonsmokers | Smokers |
| Smokers    |           |         |           |         |           |         |
| CD4\(^+\)62L\(^-\) % / \(P = .044\)/ | 7.7% | 4.0% | 86.5% | 83.7% | 5.8% | 12.4% |
| CD4\(^+\)62L\(^+\) % / \(P = .017\)/ | 54.2% | 39.6% | 40.0% | 50.0% | 5.8% | 10.4% |
| CD4\(^+\)25\(^+\) % / \(P = .013\)/ | 16.7% | 9.8% | 65.4% | 60.8% | 17.9% | 29.4% |
| CD4\(^+\)25\(^+\) abs/P / \(P = .004\)/ | 0% | 0% | 65.4% | 50.0% | 34.6% | 50.0% |
| Alcohol Consumers | Nonsmokers | Smokers | Nonsmokers | Smokers | Nonsmokers | Smokers |
| CD8\(^+\)28\(^+\) abs/P / \(P = .019\)/ | 17.3% | 10.6% | 68.3% | 64.1% | 14.4% | 25.3% |
| Ig M / \(P = .008\)/ | 0.0% | 3.2% | 90.3% | 79.5% | 9.7% | 17.3% |

Abbreviation: Ig, immunoglobulin.
exposed groups compared to controls. In intergroup analysis made by Mann-Whitney U test, a significant reduction in the relative values of the indices was recorded in third and fourth group compared to the first group ($P = .047$ and $P = .001$, respectively) and in fourth group compared to second one ($P = .014$; Table 4 and Figure 4).

A significant weak positive correlation of the relative values of the parameter with received dose ($r = .171$ at $P = .005$) and a negative one of absolute values with the length of service ($r = -.139$ at $P = .022$) were observed.

The established increase in the activated CD8+38+ subpopulation in groups with lower cumulative doses and the gradual decrease with growth up of the dose could be connected with the reaction of the activated immune system to exogenous irritants of working environment based on adaptive processes occurring in the body.

**Indicators of Humoral Immunity**

Regardless of the absence of significant differences in the number of B lymphocytes between exposed and control groups found in our previous study, the serum immunoglobulin was examined as an expression of their function. Normal distribution of results was found by Kolmogorov-Smirnov test for all 3 immunoglobulins, and Kruskal-Wallis test was applied for their analysis.

**Immunoglobulin G.** Although serum levels of immunoglobulin G did not show any statistically significant differences both between exposed groups and to control, there was some trend of increase in third group, after a drop to the control level in the first group, and in fourth group again approaching the level of control (Table 7 and Figure 5). No correlation of the study parameter was found with the received dose, age, or length of service of the respondents but a slight, positive correlation was observed for the results of IgA ($r = .187$). The frequency analysis revealed more people with elevated levels of this immunoglobulin in third group (16.9%) and in control (12.3%; Table 5).

**Immunoglobulin A.** The analysis of IgA with Mann-Whitney U test showed higher average values in all groups of professionally exposed persons compared to controls but statistically significant in third and fourth group (Table 5 and Figure 4).

Slight positive correlations of IgA with age and length of service were established ($r = .150$ and $r = .126$ at $P = .000$) as well as for IgG and IgM ($r = .187$ and $r = .259$, respectively). More of the individuals with elevated serum levels of IgA were observed in the first and third groups and in the control (Table 5).

**Immunoglobulin M.** The results for IgM was similar to that mentioned earlier—relatively higher average values compared to control in the first, second, and third groups and significant only in the first group (Table 7 and Figure 5). Frequency analysis confirmed the previous result of elevated levels of IgM observed in the first group (13.6%; Table 5).

It was found that alcohol consumption contributes to obtained deviations ($P = .008$; Table 6). Significantly weak negative correlation was established for IgM with the received dose ($r = -.112$) and a positive one for immunoglobulin A ($r = .259$).

The analysis of IgG, IgA, and IgM indicating the functional activity of B cells showed a tendency for increasing their serum concentrations in persons with cumulative dose up to 200 mSv and reducing it at higher doses to values close to control. In interpretation of data on immunoglobulin concentrations, it should be noted that the effects of other adverse factors or harmful habits might cause similar changes. In our study, it was observed that alcohol drinkers frequently have elevated levels of IgM. Japanese authors conducted a long-term study of atomic bomb survivors and also reported elevated IgM serum concentration due to compromising of the immune system that lead to some viral antigens reactivation or to the development of autoimmune processes. According to Kusunoki et al, chronic effects of low radiation doses in the range 100 to 150 mSv result in prevalence of Th2 immune response causing many diseases characterized with increased immunoglobulin production. It is not excluded that this tendency reflects the adaptation of individuals to environment factors, but it could also be the expression of humoral-type prevalence at doses above 100 mSv. A slight positive correlation of IgA with age and length of service and weak negative correlation of IgM with cumulative doses differs from our previous studies and some reports that observed a dose-dependent increase in these immunoglobulins.

On the other hand, Godekmerdan et al found significantly lower levels of serum IgG, IgA, and IgM in professionally exposed individuals compared to control group. These discrepancies could be due to both the different number of respondents and the epidemiological situation, taking into account the relatively short half-life of immunoglobulins studied.
In this survey, no direct radiation-induced changes in the immune parameters of NPP workers occupationally exposed to low doses were established. Apart from the absence of significant deviations in the normal range for the studied parameters, the observed trends in some of them do not preclude any radiation effects. There is a tendency for changes in some of observed parameters at cumulative doses 100-200 mSv which remain or approximate control values at higher doses. Relatively more manifested tendencies are observed in CD3$^+$, helper inducers cells and especially its CD4$^+$62L$^+$ subpopulation, activated/regulatory CD4$^+$25$^+$ cells, CD8$^+$28$^+$ cytotoxic subpopulation, and immunoglobulin M. Interpreting the observed trends of the studied parameters, it could be assumed that while the adaptation processes are dominated in low doses with low prevalence of Th1 immune response (increased CD8$^+$+, cytotoxic CD8$^+$+28$^+$+ and activated CD8$^+$+38$^+$+ lymphocytes) when increasing the dose above 100 to 200 mSv, the immune response shift to humoral type and compensatory mechanisms are involved to balance it (increased CD4$^+$+25$, CD4^+$+62L$^+$, and CD8$^+$+28$^+$+ lymphocytes). Although such a hypothesis could explain some of the dependencies, its proof requires additional research including the cytokine profile of cellular populations. It is known that the balance between Type 1 and Type 2 cytokines changes with age. The Th1 immune response predominates in adults and Th2 in elderly population so that the shift from Th1 to Th2 cytokine profile could be an age-associated mechanism for immune dysfunctions. The observed slight variations are hardly related only to radiation factor but to the impact of a number of other exogenous and endogenous factors on the immune system. In conclusion, it must be stressed that the significance of variations in the reference range is speculative and speak only of the existence of some trend of changes and as Fujiwara said, only 10% of them can be explained by the effect of low radiation doses.

**Declaration of Conflicting Interests**

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

**Funding**

The author(s) received no financial support for the research, authorship, and/or publication of this article.

**References**

1. Bazyka D, Chumak A, Byelyaeva N, et al. Immune cells in Chernobyl radiation workers exposed to low dose irradiation. Int J Low Rad. 2003;1(1):63-75.
2. Godekmerdan A, Ozden M, Ayar A, Ferit Gursu M, Tevfic Ozand A, Serhatioglue S. Diminished cellular and humoral immunity in workers occupationally exposed to low levels of ionizing radiation. Arch Med Res. 2004;35(4):324-328.
3. Tuschl H, Kovac R, Wottava A. Occupational exposure and its effect on some immune parameters. Int J Radiat Biol.1990;58(4): 651-659.
4. Tuschl H, Steger F, Kovac R. Occupational exposure and its effect on some immune parameters. Health Phys. 1995;68(1):59-66.
1. van Belle G, Fisher L, Heagerty PJ, Lumley T. SPSS for Windows Made Simple. East Sussex, UK: Psychology Press; 1997: 386.

2. Calgiuri G, Nicoletti A. Lymphocyte responses in acute coronary syndromes: lack of regulation spawns deviant behavior. Eur Heart J. 2006;27(21):2485-2486.

3. Kusunoki Y, Hayashi T. Long-lasting alterations of the immune system by ionizing radiation exposure: Implications for disease development among atomic bomb survivors. Int J Radiat Biol. 2008;84(1):1-14.

4. Mallat Z, Ait-Qufella H, Tedgui A. Regulatory T cell responses: potential role in the control of atherosclerosis. Curr Opin Lipid. 2005;16(5):518-524.

5. Calgiuri G, Nicoletti A. Lymphocyte responses in acute coronary syndromes: lack of regulation spawns deviant behavior. Eur Heart J. 2006;27(21):2485-2486.

6. Kusunoki Y, Hayashi T, Hakoda M, et al. Long-term effects of A-bomb radiation on the immune system: beyond a half century. RERF Update. 2004;15(1):7-18.

7. Lacoste-Collin L, Jozan S, Cances-Lauwers V, et al. Effect of continuous irradiation with a very low dose of gamma rays on life span and the immune system in SJL mice prone to B-cell lymphoma. Radiat Res. 2007;168(6):725-732.

8. Kusunoki Y, Hayashi T, Kyoizumi S. Immunity polarization in radiation and its prevention by Rad regulatory T cells for T cells. Yaku-gaku Zasshi. 1998;9(2):10-15.

9. Kusunoki Y, Hayashi T, Kyoizumi S. Immunity polarization in radiation and its prevention by Rad regulatory T cells for T cells. Yaku-gaku Zasshi. 1998;9(2):10-15.

10. Gridley DS, Asma R, Xian Luo-Owen, Adeola YM, Pecaut MJ. Low Dose, Low Dose Rate Photon Radiation Modifies Leukocyte Distribution and Gene Expression in CD4 + T. J. Radiat Res. 2009;50(2):139-150.

11. Kojima Shuji. Induction of glutathione and activation of immune function by low-dose, whole-body irradiation with γ-rays. Yakugaku Zasshi. 2006;126(10):849-857.

12. Gyuleva I, Penkova K, Rupova I, Panova D, Djounova J. Assessment of some immune parameters in occupationally exposed NPP workers I. Flow cytometry measurements of T, B, NK and NKT subset cells. Dose-Response. 2014;11(1):1-15.

13. Jackson A. Basic phenotype of lymphocytes: selection and testing of reagents and interpretation of data. Clin Immunol Newsletter. 1990;10(4):43-45.

14. Kinnear P, Gray C. Biostatistics. A methodology for the health sciences. New York, NY: John Wiley & Sons INC; 2004.

15. Van Belle G, Fisher L, Heagerty PJ, Lumley T. Biostatistics. A methodology for the health sciences. New York, NY: John Wiley & Sons INC; 2004.

16. Kanegane H, Kasahara Y, Niida Y, et al. Expression of L-selectin (CD62 L) discriminates Th1- and Th2-like cytokine-producing memory CD4 + T cells. Immunol. 1996;87(2):186-190.

17. Matsuoka S, Shinozaki K, Kobayashi N, Agematsu K. Polarization of Th1/Th2 in human CD4 + T cells separated by CD62 L: analysis by transcription factors. Allergy. 2005;60(6):780-787.

18. Adler KA, Mills PI, Dimsdale JE, Ziegler MG, Patterson TL, Sloan RP, Grant I. Temporal stability of stress-induced immune changes. Brain Behav Immun. 2002;16(3):262-274.

19. Bauer ME, Perks P, Lightman SL, Shanks N. Are adhesion molecules involved in stress-induced changes in lymphocyte distribution? Life Sci. 2001;69(10):1167-1179.

20. Gallimore A, Sakaguchi Sh. Regulation of tumor immunity by CD25 + T cells. Immunol. 2002; 107(1): 5-9.

21. Sakaguchi S. Naturally arising CD4 + regulatory T cells for immunologic self-tolerance and negative control of immune response. Annu Rev Immunol. 2004;22:531-562.

22. Damo Xu, Haiying Liu, Mousa Komai-Koma, Carol Campbell, Charlie McSharry. CD4 + CD25 + Regulatory T Cells Suppress Differentiation and Function of Th1 and Th2 Cells, Leshmania major Infection, and Colitis in Mice. J Immunol. 2003;170(1):394-399.

23. Sakaguchi N, Miyai K, Sakaguchi S. Ionizing radiation and autoimmunity. Induction of autoimmune disease in mice by high dose fractionated total lymphoid irradiation and its prevention by inoculating normal T cells. J Immunol. 1994;152(5):2586-2595.

24. Xu Y, Greenstock CL, Trivedi A, Mitchell RE. Occupational levels of radiation exposure induce surface expression of IL-2 receptors in stimulated human peripheral blood lymphocytes. Rad Environ Biophys. 1996;35(2):89-93.

25. Liu SZ, Shun-Zi J, Xiao-Dong L, Yi-Min S. Role of CD28/B7 costimulation and IL-12/IL-10 interaction in the radiation-induced immune changes. BMC Immunol. 2001;2(8):1471-2172.

26. Torkabadi E, Kariminia A, Zak`eri F. Alteration of peripheral blood T-reg cell and cytokines production in angiography personnel exposed to scattered x-rays. Iran J Allergy Asthma Immunol. 2007;6(4):181-187.

27. Kusunoki Y, Hayashi T. Long-lasting alterations of the immune system by ionizing radiation exposure: Implications for disease development among atomic bomb survivors. Int J Radiat Biol. 2008;84(1):1-14.

28. Mallat Z, Ait-Qufella H, Tedgui A. Regulatory T cell responses: potential role in the control of atherosclerosis. Curr Opin Lipid. 2005;16(5):518-524.

29. Calgiuri G, Nicoletti A. Lymphocyte responses in acute coronary syndromes: lack of regulation spawns deviant behavior. Eur Heart J. 2006;27(21):2485-2486.

30. Nerisshi K, Nakashima E, Delongchamp RR. Persistent subclinical inflammation among A-bomb survivors. Int J Radiat Biol. 2001;77(4):475-482.

31. Bogdanyki EN, Balogh A, Felgyinszki N, et al. Effects of low-dose radiation on the immune system of mice after total-body irradiation. Radiat Res. 2010;174(4):480-489.

32. Pandey R, Shankar B, Sharma D, Sainis K. Low dose radiation induced immunomodulation: effect on macrophages and CD8 + T cells. Int J Radiat Biol. 2005;81(11):801-812.

33. 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(5):497-508.

34. Fujiwara S, Carter RL, Akiyama M, et al. Autoantibodies and immunoglobulins among atomic-bomb survivors. Radiat Res. 1994;137(1):89-95

35. Sandmand M, Bruunsgaard H, Kemp K, et al. Is ageing associated with a shift in the balance between Type1 and Type2 cytokines in humans? Clin Exp Immunol. 2002;127(1):107-114.