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ASSESSMENT OF SOME IMMUNE PARAMETERS IN OCCUPATIONALLY EXPOSED NUCLEAR POWER PLANTS WORKERS: FLOWCYTOMETRY MEASUREMENTS OF T, B, NK AND NKT CELLS

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□ The purpose of this article is to analyze the results of a 10-year survey of the radiation effects of some immune parameters of occupationally exposed personnel from the Nuclear Power Plant "Kozloduy", Bulgaria. 438 persons working in NPP with cumulative doses between 0.06 mSv and 766.36mSv and a control group with 65 persons were studied. Flow cytometry measurements of T, B, natural killer (NK) and natural killer T (NKT) cell lymphocyte populations were performed. Data were interpreted with regard to cumulative doses, length of service and age. The average values of the studied parameters of cellular immunity were in the reference range relative to age and for most of the workers were not significantly different from the control values. Low doses of ionizing radiation showed some trends of change in the number of CD3+CD4+ helper-inducer lymphocytes, CD3+ CD8+ and NKT cell counts. The observed changes in some of the studied parameters could be interpreted in terms of adaptation processes at low doses. At doses above 100–200 mSv, compensatory mechanisms might be involved to balance deviations in lymphocyte subsets. The observed variations in some cases could not be attributed only to the radiation exposure because of the impact of a number of other exogenous and endogenous factors on the immune system.

Keywords: Immunology, ionising radiation, low-dose effects, lymphocytes, flowcytometry

INTRODUCTION

The effect of radiation on the immune response has become one of the chief research fields in radiation biology and radiation protection. The immune system is considered to be critically affected in cases of acute radiation exposure accounting for doses above 100 mSv. Occupational and environmental illnesses are often associated with alterations in the immune system, which may be manifested through changes in the proportions of lymphocyte subpopulations.

The radiosensitivity of different lymphocyte subsets depends on many factors such as degree of differentiation, state of activation, spontaneous apoptosis of the subset etc. Although B-lymphocytes are considered to be

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the most radiosensitive cells and natural killer (NK) cells, the most radio resistant ones in vivo (Mori *et al.* 2005, Schmitz *et al.* 2003), taking into-account the spontaneous apoptosis of different lymphocyte populations, CD8+Tcells appeared to be the most radiosensitive (Wilkins *et al.* 2002b) followed by CD4+benchmark studies.

The response of humans to low and moderate doses radiation is quite unclear, as the sample size of the population that has been studied is still relatively small and more of them are focused on changes in immunity after exposure to doses higher than 0.2 Gy. Most of what is known about radiation effects and risks comes from animal studies, a few industrial radiation accidents(Bazyka *et al.* 2003, Kusmenok *et al.* 2003), residents of contaminated areas (Chang *et al.* 1999, Sajjadieh *et al.* 2009) or data from Hiroshima and Nagasaki bombings (Akiyama 1995, Kusunoki *et al.*1998, Kusunoki *et al.* 2004, Kusunoki and Hayashi 2008). Although the observed populations have been exposed to ionizing radiation in very different conditions there is some similarity in long-lasting alterations of the immune system, expressed mainly in an impairment of T-cell immunity, decreased total T cell counts and CD4+ T cell counts, increased humoral immunity and a shift towards an inflamatory profile.

There is much controversy about the effects of chronic low-dose exposure to ionizing radiation and the possible consequences particularly in occupational exposure. The reports specifically concerning the immune status of occupationally exposed persons (Godekmerdan *et al.* 2004, Korraa *et al.* 2010, Rees *et al.*2004, Torkabadi *et al.* 2007, Tuschl *et al.* 1990, 1995, Zakeri *et al.* 2010) are quite limited and not uniform. It is difficult to identify whether the observed effects are associated only with the received dose, which often does not exceed the natural background level, or other occupational and environmental factors are also involved.

The aim of this study was to investigate the effect of occupational exposure to low and moderate levels of ionizing radiation in nuclear power plant (NPP) workers over a 10-year period by selected indices of cellular immunity.

MATERIALS AND METHODS

The group of NPP workers occupationally exposed to low dose radiation consists of 438 persons (421 male and 17 female) and the control group consists of 65 persons without any work-related exposure to ionizing radiation (49 male and 16 female) with similar age, sex and length of employment. The study was performed within the framework of a bilateral contract between the National Centre of Radiobiology and Radiation Protection of the Ministry of Health of Bulgaria and the NPP Kozloduy, aiming to study the impact of occupational chronic low-dose exposure on immunological and health status of workers. Informed consent was obtained from all participants.

The group of occupationally exposed individuals was selected from the same workplace (service personnel of units 5 and 6 of NPP Kozloduy) because of radiation inhomogeneity of different locations in the plant. The radiation doses were determined by NPP individual exposure monitoring program.

For the purposes of our research the workers were divided in groups according to 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 completing a questionnaire, the participants were subjected to medical examinations and underwent basic hematological assay for health status evaluation. No deviations in the basic laboratory tests and current infections were found in the respondents. Some of them were diagnosed with cardiovascular diseases (hypertension, ischemic heart disease), obesity, diagnosed not chronic hepatitis 10 years ago, and other chronic diseases, whose distribution among the groups is illustrated in Table 2.

Blood was collected from each participant by venipuncture, into Vacationer EDTA tubes (Greiner Bio-One GmbH, Kremsmunster, Austria). Total white blood cells (WBC) and lymphocytes were counted using an automatic haematology analyser ABX Pentra 60 C+ (HORIBA GROUP ABX Diagnostics, Montpellier, France) operated in CBC + 5DIFF (Cell Blood Count + 5 population differential count) modes.

Blood smears were prepared for WBC morphological observation. The samples were processed and stained within 6 h of blood collection. Peripheral blood lymphocyte subsets were determined by two-colour flow-cytometric analysis using a panel of fluoroscein isothiocyanate (FITC)- and phycoerythrin (PE)-)-conjugated anti-CD monoclonal antibodies (mAbs) (Becton Dickinson Biosciences, San Jose, California, USA). Direct two-colour immunofluorescence was performed in whole blood by the method of Jackson (1990) as described in Becton Dickinson

TABLE 1. NPP personnel: groups according to received doses, average age and length of employment.

Personnel					
Group	Dose (mSv)	n	Average age (years)*	Length of service (years)*	Received dose (mSv)*
Control	0	65 (49 male and 16 female)	46 ± 8	19 ± 8	0
1	0.1–20	103 (93 male and 10 female)	43 ± 7	16 ± 8	9.53 ± 5.8
2	20.1–100	117 (112 male and 5 female)	42 ± 8	16 ± 8	49.42 ± 21.7
3	100.1–200	66 (64 male and 2 female)	45 ± 7	18 ± 6	141.95 ± 30.4
4	>200	153 (male)	45 ± 6	20 ± 6	354.48 ± 120.3

*Indicate the standard deviation (±SD).

TABLE 2. General health status of NPP workers

		Groups*				
Condition	Health status	Control (%)	0.1–20 mSv (%)	20–100 mSv (%)	100–200 mSv (%)	> 200 mSv (%)
Mobius hypertonicus	healthy	81.8	66.7	64.5	68.4	62.1
	with disease	18.2	33.3	35.5	31.6	37.9
Cardio vascular diseases	healthy	78.8	66.7	64.5	63.2	62.1
	with disease	21.2	33.3	35.5	36.8	37.9
Obesities	healthy	84.8	85.2	74.2	57.9	93.1
	with disease	15.2	14.8	25.8	42.1	6.9
Diabetes mellitus	healthy	97.0	92.6	96.8	100.0	96.6
	with disease	3.0	7.4	3.2	-	3.4
Hepatitis	healthy	97.0	88.9	90.3	89.5	93.1
(inactive. not chronic)	with disease	3.0	11.1	9.7	10.5	6.9
Vaccinations and virus	no	84.8	85.2	93.5	89.5	89.7
diseases in last month	yes	15.2	14.8	6.5	10.5	10.3
Gastrointestinal diseases	healthy	89.2	93.6	93.4	94.9	94.1
	with disease	10.8	6.4	6.6	5.1	5.9
Pulmonary diseases	healthy	100.0	96.8	97.8	96.6	97.4
	with disease	-	3.2	2.2	3.4	2.6

*Data are presented as percent of all individuals in each group.

Monoclonal Antibodies Source Book. Simultest IMK-Lymphocyte kit and BD Simultest CD57 FITC/CD8 PE (Becton Dickinson Biosciences, San Jose, California, USA) were used. Approximately 10^4 events per sample were analyzed with FACS Calibur flow cytometer. 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. FACS Calibur flow cytometer, Simulset and CellQuest softwares (Becton Dickenson Biosciences, San Jose, CA) were used for data analysis.

The following lymphocyte subsets as percentages of the lymphocyte count were examined: CD3+(total T lymphocytes-responsible for cell-mediated immune response of adaptive immunity), CD19+ (B lymphocytes-responsible for production of antigen-specific antibody to extracellular pathogens in response to infection), CD4+ (helper-inducer T lymphocytes- recognize antigen presented by MHC II molecules and regulate immune response as by cytokines production facilitate interaction between T and B lymphocytes and macrophages. CD4+ T lymphocytes are divided in two types- inflammatory cells (Th1) activates macrophage to destroy intracellular microorganisms and helper cells (Th2) helps B cells to differentiate and secrete immunoglobulin), CD8+ (cytotoxic-suppressor T lymphocytes-recognize cells infected with viruses by complex of viral fragment with MHC class I. CD8+ are effectors T cells with cytotoxic activity against viruses, tumor cells and fungus.), CD3-CD16+CD56+ (NK

cells-Natural Killer cells, exhibiting a non-MHC restricted cytotoxicity involved in anti- tumor and anti-viral immunity and mediate antibody-dependent cellular cytotoxicity), CD3+CD16+CD56+ (natural killer T [NKT] cells- T lymphocytes with NK similar activity, occupying an intermediate role between innate and acquired immunity, and involved in the regulation of adaptive immune response against its own and foreign antigens), CD57+CD8- (subclass phenotype of NK cells, pick primarily in intracellular infections) and CD57+CD8+ (T lymphocytes with NK-like activity, pick in presence of persistent intercellular antigenic irritation). The reference values for the Bulgarian population defined by the Central Laboratory of Clinical Immunology, University Hospital "Alexandrovska" (Sofia) are listed in Table 3. There are no results about sex-dependent differences in the studied age range for the Bulgarian population.

Statistical methods

The following statistical methods were applied to process the results.

1. Variation analysis of quantitative variables - mean, standard deviation, standard error of the mean and 95% confidence interval of the mean. Statistical analysis was performed using parametric and nonparametric methods as follows:
   a. Parametric methods - one-way analysis of variance (ANOVA) to check the equality of more than two mean values in a normal distribution.
   b. Nonparametric methods - Kolmogorov–Smirnov and Shapiro–Wilk tests to check the normality of distribution of quantitative variables; Mann–Whitney test for comparison of averages in two groups of one quantitative variable when the distribution is not normal; Kruskal–

TABLE 3. Reference values* for lymphocyte subsets as percentage of lymphocyte count

Indices	Percentage values (%)	Absolute values ($\times 10^9$)
CD3+	61–85	1000–2000 cells/μl
CD3+4+	34–59	560–1410 cells/μl
CD3+8+	19–36	320–960 cells/μl
CD3+4+/CD3+8+	-	1.1–2.8
CD19+	6–15	110–390 cells/μl
CD3-16+56+	7–26	110–620 cells/μl
CD3+CD16+CD56+	3–10	44–356 cells/μl
CD57+CD8-	3–13	44–400 cells/μl
CD57+CD8+	5–11	9–257 cells/μl

*Reference values for the Bulgarian population is 5th percentile to 95th percentile range by data of Central Laboratory of Clinical Immunology, University Hospital Alexandrovska,Sofia, Bulgaria (Naumova E and Altankova I. 2001)

Wallis test for comparison of averages in more than two groups of quantitative variables when the distribution is not normal.
2. Frequency analysis - the frequencies of observations occurring in certain ranges of values
3. Correlation analysis - coefficient of linear correlation, parametric (Pearson) and non-parametric (Spearman).

SPSS v. 11.0.1 for Windows was used for data processing (Kinnear and Gray 1997, Van Belle *et al* 2004).

RESULTS

The relative and absolute values of cell parameters were analyzed. Additional factors such as smoking, consumption of alcohol and morbidity were taken into account in interpreting data. The percentage (mean %±SD) of lymphocytes subsets, as a percentage of lymphocyte count are given in Table 4.

Our results revealed that most changes in the main lymphocytes subsets fluctuate within the lower and upper reference range of the relevant parameter for the middle-aged Bulgarian population.

No significant difference was found by ANOVA parametric analysis in the average relative number of B lymphocytes (CD19+) and total T lymphocytes (CD3+) compared to the control and between the groups (Table 4). The observed slight increase in B lymphocytes and decrease in T lymphocytes mean values in groups with doses over 100 mSv was not statistically significant.

The nonparametric Kruskal–Wallis test did not reveal statistically significant differences among any of the groups of responders in the NK cell (CD3-CD16+CD56+) counts and in CD57+CD8- phenotype, although a higher mean value for CD57+CD8- phenotype was recorded in the group with cumulative dose below 20 mSv (Table 4).

As T lymphocytes are heterogeneous, an extensive analysis of their main subpopulations was made (Table 4). Lower average relative values of CD4+ T lymphocytes were observed in groups with cumulative doses below 200 mSv. Statistical significance was established by nonparametric Mann–Whitney analysis in groups exposed to doses 0.1 – 20 mSv and to 100.1 – 200 mSv. Increase of the mean values of CD3+CD8+ cytotoxic-suppressor T lymphocytes was found in the same groups, but statistical significance was found only for the relative values in the group exposed to doses below 20 mSv (Table 4). The mean values of CD4+/CD8+ index representing the ratio between helper-inducer and cytotoxic-suppressor lymphocytes also show a significant decrease in groups exposed to doses of 0.1 –20 mSv and 100.1 – 200 mSv.

Higher mean values in NKT cells CD3+CD16+CD56+ and CD57+CD8+ T lymphocytes counts were observed (Table 4). An increased number

TABLE 4. Variation analysis of percentage of B, total, helper-inducer, cytotoxic-suppressor T lymphocyte and NK and NKT cell in NPP workers

Parameters, Groups, mSv	B lymphocytes (CD19+) X ± SD (%)	Total T lymphocytes (CD 3+) X ± SD (%)	Helper-inducer T lymphocytes (CD3+CD4+) X ± SD (%)	Cytotoxic-suppressor T lymphocytes (CD3+CD8+) X ± SD (%)	CD4+/CD8+ ratio X ± SD	NK cells (CD3-CD16+ CD56+) X ± SD (%)	NK cells with phenotype (CD57+CD8-) X ± SD (%)	NKT cells (CD3+CD16+ CD56+) X ± SD (%)	T lymphocytes with NK activity (CD57+CD8+) X ± SD (%)
Control 0.0	9.80 ± 3.4	72.08 ± 5.9	44.19 ± 6.5	26.34 ± 6.7	1.78 ± 0.6	16.58 ± 6.0	6.67 ± 4.8	5.48 ± 5.1	12.45 ± 5.2
Group 1 0.1 – 20	9.72 ± 3.7	71.65 ± 8.3	40.35 ± 9.0 **P < 0.016**	29.66 ± 8.9 **P < 0.005**	1.54±0.76 **P < 0.000**	16.90 ± 7.8	8.11 ± 6.4	7.94 ± 7.4 **P = 0.004**	17.78 ±7.9* **P < 0.000**
Group 2 20.1–100	9.98 ± 4.2	70.24 ± 8.1	41.33 ± 7.9	26.86 ± 8.3	1.72 ± 0.7	17.79 ± 7.8	6.76 ± 4.1	8.94 ± 8.3 **P= 0.006**	16.03 ± 7.8 **P < 0.010**
Group 3 100.1 –200	10.56 ± 4.8	69.42 ± 9.4	39.67 ± 9.3 **P < 0.015**	27.98 ± 9.9	1.63 ± 0.8 **P = 0.021**	17.38 ± 8.5	6.98 ± 4.4	8.27 ± 7.9	16.83 ± 9.4 **P < 0.031**
Group 4 >200	10.09 ± 4.4	70.93 ± 7.9	42.31 ± 7.7	26.38 ± 8.1	1.78 ± 0.7	16.79 ± 7.2	6.73 ± 4.5	6.77 ± 6.7	15.54 ± 7.3* **P < 0.005**

*Significant difference in the average relative values of (CD57+ CD8+) was found between Group 1 and Group 4 ($P = 0.040$).
Statistically significant differences compared with the control group are marked with bold type.

of cells with phenotype CD3+CD16+CD56+ were found in groups with cumulative doses below 200 mSv, where at doses lower than 100 mSv was statistically significant (Table 4). In the group with cumulative doses above 200 mSv the values had decreased to controls. A marked statistically significant increase in the average counts of CD57+CD8+ T lymphocytes with NK activity was established in all exposed groups by Kruskal–Wallis test (Table 4). The comparison between exposed groups with nonparametric Mann–Whitney analysis revealed statistical difference (p=0.040) in the relative values of this parameter in groups with doses below 20mSv and in the group with doses over 200mSv.

The frequency analysis (Table 5) confirmed the observed trend for the lymphocytes subsets established by variation analysis. A higher frequency of individuals with values above the reference range was found in all exposed groups for B lymphocytes, NK and NKT cells, with phenotype CD57+CD8+. A higher number of persons showed counts above the reference for suppressor-cytotoxic T lymphocytes and NKT CD3+56+16+ cells mainly in groups with cumulative doses below 20 mSv and between 100.1–200 mSv.

Contrary, a higher frequency of individuals with lower values of total and helper-inducer T lymphocytes, and CD4+/CD8+ ratio was observed in groups with doses from 20 to 200 mSv.

The correlation analysis of the studied parameters for cumulative dose, age and length of employment revealed weak correlation coefficients in most cases. No significant correlation was found between any of lymphocytes subsets and the doses received. A weak negative correlation with age for the absolute values of B-lymphocytes ($r = -0.089$; $p<0.05$ by Spearman) and for the relative values of CD57+CD8- NK cells ($r = -0.106$; $p<0.032$ by Spearman) was recorded. Whereas, the relative values of helper-inducer T lymphocytes and NKT CD3+56+16+ cells were weakly positive related with age ($r = 0.108$; $p = 0.027$ by Pearson) and ($r = 0.105$; $p = 0.043$ by Spearman), respectively. The relative values of total and of helper-inducer T lymphocytes were slightly related to length of employment ($r = 0.137$; $p = 0.005$ by Pearson) and accordingly ($r = 0.136$; $p = 0.005$ by Pearson).

Analysing the effects of other adverse factors, the present survey did not establish any dependence of the studied parameters on alcohol consumption (Table 6). Considering the effect of smoking, it was found that the number of smokers with deviations was significantly lower than that of non-smokers for helper-inducer ($p = 0.026$), suppressor-cytotoxic ($p = 0.026$) T lymphocytes, and T lymphocytes with NK activity ($p=0.023$). Only for B lymphocytes was observed that smokers showed a higher percentage with values out of range than non-smokers ($p=0.025$).

TABLE 5. Frequency analysis of studied immune parameters in NPP workers given as a percentage of the number of subjects, who had cell counts under, within or above reference range

Parameters	Dose (mSv)	Under the reference range (%)	Within the reference range (%)	Above the reference range (%)
1	2	3	4	5
CD 19+	0 (Control)	7.8	87.5	4.7
	0.1 – 20	12.6	80.6	6.8
	20.1 – 100	12.0	77.8	10.2
	100.1 – 200	10.6	75.8	13.6
	>200	14.4	75.2	10.4
CD 3+	0 (Control)	3.1	96.9	-
	0.1 – 20	11.7	85.4	2.9
	20.1 – 100	13.7	84.6	1.7
	100.1 – 200	15.2	83.3	1.5
	>200	10.5	86.9	2.6
CD4/CD8 index	0 (Control)	9.6	86.2	4.2
	0.1 – 20	35.3	52.3	12.7
	20.1 – 100	19.7	67.5	12.8
	100.1 – 200	30.3	60.6	9.1
	>200	17.0	71.2	11.8
CD3+ CD4+	0 (Control)	3.2	95.4	1.4
	0.1 – 20	28.2	68	3.8
	20.1 – 100	16.2	81	2.6
	100.1 – 200	24.2	75	
	>200	9.8	87.6	2.6
CD3+ CD8+	0 (Control)	4.6	90.8	4.6
	0.1 – 20	13.6	69.9	16.5
	20.1 – 100	17.1	67.5	15.4
	100.1 – 200	13.6	68.2	18.2
	>200	17.6	73.2	7.2
NK (CD3- CD16+ CD56+)	0 (Control)	6.2	83.0	10.8
	0.1 – 20	9.7	72.8	17.5
	20.1 – 100	8.5	77.0	14.5
	100.1 – 200	4.5	77.3	18.2
	>200	8.5	73.9	17.6
NKT (CD3+ CD16+ CD56+)	0 (Control)	0	85.4	14.6
	0.1 – 20	0	77.8	22.2
	20.1 – 100	0	73.7	26.3
	100.1 – 200	0	72.7	27.3
	>200	0	82.7	17.3
NK (CD57+ CD8-)	0 (Control)	6.6	88.5	4.9
	0.1 – 20	6.4	74.5	19.1
	20.1 – 100	4.2	93.1	2.8
	100.1 – 200	3.7	88.9	7.4
	>200	13	77.2	9.8
NKT (CD57+ CD8+)	0 (Control)	0	85.4	14.6
	0.1 – 20	0	77.8	22.2
	20.1 – 100	0	73.70	26.3
	100.1 – 200	0	72.7	27.3
	>200	0	82.7	17.3

TABLE 6. Immune parameters changes in smoking and non-smoking persons

	Under reference range %		Reference range %		Above reference range %	
Parameters	Non smokers	Smokers	Non smokers	Smokers	Non smokers	Smokers
CD3+4+ % /p =0.026/	**23.5**	**14.2**	75.9	82.8	0.6	2.9
CD3+8+ % /p =0.023/	10.5	13.7	69.1	76.0	**20.4**	**10.3**
CD19+ abs/p=0.025/	12.3	7.9	82.7	79.9	**4.9**	**12.3**
CD57+8+%/p=0.003/	3.8	1.5	16.8	31.0	**79.4**	**67.5**

Statistically significant differences compared with the control group are marked with bold type.

DISCUSSION

In the present study no significant immunological effects were observed in the surveyed NPP workers when compared to a reference population. The obtained results demonstrated that the average values of the studied cellular immunity parameters in NPP workers were within the age-specific reference range for Bulgarian population (Baltadjieva *et al* 2003). The revealed correlations to cumulative dose, length of employment and age were weak and could rather be considered as trends in the studied cellular immunity parameters.

No significant differences were found between the exposed groups and the control in terms of the main lymphocyte populations - B and T lymphocytes, NK cells with phenotypes CD3-CD16+ CD56+ and CD57+ CD8-. Similar observations have been reported by other authors who did not find changes of the total T cell, B cell and NK cell counts in occupationally exposed persons (Godekmerdan *et al.* 2004, Rees *et al.* 2004, Zakeri *et al.* 2010). The weak inverse correlation of B lymphocyte counts with age in our study is in good correspondence with some investigations for an age-related decline of this cell population in peripheral blood (Hakim *et al.* 2004, Huang *et al.* 2005, Weng 2006).

Our results for NK cells with phenotypes CD3-CD16+ CD56+ and CD57+ CD8- are in agreement with other reports suggesting that NK cells are one of the most radio resistant cell populations (Akiyama 1995; Kusunoki *et al.* 1998; Schmitz *et al.* 2003; Kusunoki *et al.* 2004; Mori *et al.* 2005; Zakeri *et al.* 2010). The higher frequency of subjects with values above the reference range observed by us in all groups could be considered as an age-related increase in these cell counts, since most of the individuals who took part in our study are in the age range of 45±7. Recent studies (Hakims *et al.* 2004; Huang *et al.* 2005; Weng *et al.* 2006; Tamura and Ogata 2009) revealed that an increase of NK and NKT cells in the elderly population may be considered as a marker of successful aging.

The deviations observed in the T cell subsets are expressed by some decrease in the relative values of CD4+T lymphocytes and increase in CD8+ T lymphocytes up to a cumulative dose of 200 mSv. The changes obtained for CD4/CD8 index seemed to reflect changes found in helper-inducer lymphocytes which correspond well with *in vitro* studies suggesting that the CD4+/CD8+ ratio and CD4+ T cells affect the apoptotic response of human lymphocytes to ionizing radiation (Wilkins *et al.* 2002a).

Our findings are in agreement with the results of Godekmerdan *et al. (*2004), who reported significantly lower CD4+T lymphocyte counts in radiology workers. Chang *et al.* (1999) also found reduced CD4+ T lymphocyte counts and CD4+/CD8+ ratios and moderately increased CD8+ T cell counts in residents in a building with radioactive source. Other authors (Rees *et al.* 2004, Torkabadi *et al.* 2007, and Zakeri *et al.* 2010) did not find any difference in the rate of CD4+, CD8+ T cells of occupationally exposed persons. Contrary to our results, Tuschl *et al.* (1990, 1995) observed a reduction in CD8+ T-cell counts. Such inconsistency may be due to the different exposure rates and duration of exposure, or to variations in the immune status of the subjects studied and the different in the number of the study population.

It is difficult to understand the mechanisms for the different level of radiosensitivity of T lymphocyte subsets to low-dose exposure. Furthermore, the rate of spontaneous and radiation-induced apoptosis differs in all lymphocyte subpopulation and between individuals (Schmitz *et al.* 2003). The delayed CD4+ subset recovery which is only thymus dependent and impaired in older population, could also contribute to the slight reducing of T helper-inducer cells.The observed significant increase in mean values of CD8+ lymphocytes in the group with cumulative doses below 20 mSv may be explained with the study of Bogdandi *et al.* (2010) finding that CD8+ and B cells are rather resistant to low doses, as those in occupational exposures.

Our observation of the gradually increasing average counts of NKT cells with phenotypes CD3+CD16+CD56+ and NK-like T cells CD57+CD8+ up to doses of 200 mSv could be regarded as an expression of stimulating innate immunity. This assumption arises from the intermediate role of these cells between innate and acquired immunity and the suggestion that low-dose radiation could stimulate the innate immunity (Ren *et a.l* 2006). The recorded in our investigation significantly higher count of NKT cells in the exposed groups could be considered as a favorable physiological and immunological reaction to the enviromentals factors (Faunce and Palmer 2009). Similar results are reported for an augmentation of NKT cells in Chernobyl clean-up workers as a late effect of the Chernobyl accident (Kusmenok *et al.* 2003). The observed slight trend in increasing the number of NKT cells with age and the higher frequency of persons with values above the reference in all groups indicate age-related dependence

(Ohkawa *et al.* 2001). It is considered (Faunce and Palmer 2009) that the increase in the number of NKT cells in aging is associated with a defective functional capacity, particularly cytokine production and a Th1 to Th2 shift in their cytokine response profile (Jing *et al.* 2007).

Most pronounced significant variations in the studied parameters were observed in the group of workers with cumulative doses below 20 mSv where was recorded a higher incidence of individuals with above reference values for CD8, NK and NKT, and a lower incidence than the reference for CD4 and CD4/CD8. The established in this group higher percentage of people with clinical history of hepatitis, not active at the time of the study, and a relatively high number of individuals with a recent influenza vaccine might contribute for the observed results (Table 2).

The analysis of the effect of morbidity on the immune status of the studied cohort (Table 2) indicated an increased frequency of hypertension, obesity and cardiovascular diseases in all examined groups. The observed trend for significant reduction of CD4+ lymphocytes average values may contribute to risk of various diseases, such as arteriosclerosis, hypertension and coronary heart disease. Kusunoki *et al.* (1999) found significant association between a lower proportion of CD4+ T cells and myocardial infarction in atomic bombing survivors and assumed a causal relationship between immune dysfunction and cardiovascular diseases. Interpreting the morbidity in the respondents we could accept the assumption of Kusunoki Y and Hayashi (2008) and Kusunoki *et al.* (2010) that radiation-induced T-cell immunosenescence may result in activation of inflammatory responses and may be partly involved in the development of ageing-associated and inflammation-related diseases.

In conclusion, the results of this study showed a more pronounced tendency for changes in CD4+, CD8+, NKT cell counts and CD4+/CD8+ ratio, which could be associated with the cumulative dose and the age. The differences in the studied parameters observed in the exposed persons increased up to cumulative doses of 200 mSv, whereas at doses higher than 200 mSv, the values reached a plateau or even went to those of the control group. It may be suggested that while the observed tendency at lower doses could be associated with adaptive response, at higher doses other compensatory mechanisms might be included. Data of Kusunoki *et al.* (2001), Kusunoki *et al.* (2004) about a radiation-induced imbalance between Th1 and Th2 pathways, switching toward a Th2 profile, and our results for reversal trends in some lymphocyte subpopulations at doses above 200 mSv, give us a reason to suppose a similar pattern in the studied contingent. Likewise, it should be kept in mind that the balance between Type 1 and Type 2 cytokines changes with age so that the shift from a Th1 to a Th2 cytokine profile could be an age-associated compensatory mechanism against immune dysfunctions (Sandmand *et al.* 2002). Further studies should be made, including additional lymphocyte subpopulations

and cytokines in order to confirm this assumption and to clarify the mechanisms underlying the low-dose occupational exposure effects on the immune parameters.

CONCLUSIONS

The immulogical study of NPP workers occupationally exposed to low doses of ionizing radiation showed some trends of change in the number of CD3+CD4+ helper-inducer lymphocytes, CD3+ CD8+ and NKT cell counts as compared to the control group. The observed variations in some cases could not be attributed only to the radiation exposure because of the impact of a number of other exogenous and endogenous factors on the immune system.

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