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ORIGINAL PAPER

Expression of blood serum proteins and lymphocyte differentiation clusters after chronic occupational exposure to ionizing radiation

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Abstract This study aimed to assess effects of chronic occupational exposure on immune status in Mayak workers chronically exposed to ionizing radiation (IR). The study cohort consists of 77 workers occupationally exposed to external gamma-rays at total dose from 0.5 to 3.0 Gy (14 individuals) and workers with combined exposure (external gamma-rays at total dose range 0.7–5.1 Gy and internal alpha-radiation from incorporated plutonium with a body burden of 0.3–16.4 kBq). The control group consists of 43 age- and sex-matched individuals who never were exposed to IR, never involved in any cleanup operations following radiation accidents and never resided at contaminated areas. Enzyme-linked immunoassay and flow cytometry were used to determine the relative concentration of lymphocytes and proteins. The concentrations of T-lymphocytes, interleukin-8 and immunoglobulins G were decreased in external gamma-exposed workers relative to control. Relative concentrations of NKT-lymphocytes, concentrations of transforming growth factor-β, interferon gamma, immunoglobulins A, immunoglobulins M and matrix proteinase-9 were higher in this group as compared with control. Relative concentrations of T-lymphocytes and concentration of interleukin-8 were decreased, while both the relative and absolute concentration of natural killers, concentration of immunoglobulins A and M and matrix proteinase-9 were increased in workers with combined exposure as compared to control. An inverse linear relation was revealed between absolute concentration of T-lymphocytes, relative and absolute concentration of T-helpers cells, concentration of interferon gamma and total absorbed dose from external gamma-rays in exposed workers. For workers with incorporated plutonium, there was an inverse linear relation of absolute concentration of T-helpers as well as direct linear relation of relative concentration of NKT-lymphocytes to total absorbed red bone marrow dose from internal alpha-radiation. In all, chronic occupational IR exposure of workers induced a depletion of immune cells in peripheral blood of the individuals involved.

Keywords Gamma-rays · Alpha-radiation · Lymphocytes · Immunoglobulins · Cytokines

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Introduction

Being one of the most radiosensitive human organ systems, the immune system plays a critical role in the pathogenic mechanisms of late effects induced by exposure to ionizing radiation (IR). Thus, close attention is paid to studies on IR-induced damages.

A large number of studies in this area are devoted to acute radiation effects on animals (Courtade 2001; Liu et al. 2003); acute exposure in Hiroshima and Nagasaki populations (Akiyama 1995; Kusunoki et al. 1998, 1999, 2010; Hayashi et al. 2003, 2005; Neriishi et al. 2001); chronic combined exposure (Akleyev 2007, 2012; Ovcharova 2006; Tahauov et al. 2003, 2005; Voronova 2007;

Kirillova et al. 2012); and effects of chronic external gamma-rays on human immune status (Rees et al. 2004).

However, effects of low and moderate doses from external gamma-rays in the delayed period after exposure are rarely studied. In addition, not all of parameters of immune and cytokine status were studied in workers with combined exposure, so it calls for further research.

It was shown that in terms of acute and chronic exposure apoptosis of immunocompetent progenitor cells, cytokine balance shift to proinflammatory profile, mutations in T-cell receptor (TCR), bystander effect, genome instability, acceleration of immunosenescence, modification of antigen presentation, autoimmune disorders and immune homeostasis disorders may be possible mechanisms of immune disorders (UNCEAR 2006).

Radiation-induced apoptosis, a key mechanism of radiation damages, is well studied in lymphocytes. At least two ways of radiation-induced apoptosis are known, the one is mediated by mitochondrial factors and is p53 dependant, the other is mediated by cell surface receptors Fas/CD95 (Shankar and Sainis 2005; Kolesnik and Fux 2003).

Gene mutations of TCR are dose-dependant mechanism leading to formation of defective TCRs and to self–nonself discrimination (Kyoizumi et al. 1992; Umeki et al. 1997).

There are a lot of conflicting data on cytokines secreted into the blood due to radiation exposure, but most likely, their balance is shifted to the proinflammatory profile (Nikolic et al. 2000; Schaue et al. 2012). Inflammation is supposed to be one of the mechanisms of delayed effects of exposure, such as pneumofibrosis, cancer and cardiovascular pathologies (Westermann et al. 1999; Kusunoki et al. 2004; Nakachi et al. 2004).

Such delayed effects as genome instability and bystander effect were revealed after radiation exposure to cells of the immune system (Duhrsen and Metcalf 1990; Greenberger et al. 1996).

Some studies shown that after radiation exposure cells of the immune system have abnormalities similar to those observed during physiological aging (Kusunoki et al. 1998). Changes in antigen presentation, probably, indicate to immunosenescence due to ionizing radiation (Liao et al. 2004).

Ionizing radiation may induce failure of self-tolerance and autoimmune disorders (Oldstone 1998).

Experimental and epidemiological data show that radiation affects homeostasis of T cells, i.e., decreases the ability of the immune system to produce new naïve T cells and reduces the repertoire of TCRs memory T cells. This may lead to low immunity against new pathogens and to decreased ability to control recurrent and latent infections (Yamaoka et al. 2004; Kusunoki et al. 2003).

Chronic exposure is characterized by prolonged ionizing radiation when effects as cell structure damages and adaptive processes run in parallel (Akleyev 2012). Experimental studies of chronic exposure in mice revealed immunosuppression expressed in the reduced pool of pluripotent lymphocytes precursors, stable moderate hypoplasia of central and peripheral organs of the immune system, inhibition of antibody synthesis occurring long after the end of irradiation (Kirillova et al. 1988). However, elsewhere (Ishioka et al. 1997; Pandey et al. 2005), on the contrary, there is evidence of immune reactions activation in mice exposed to low-dose rate of chronic or fractionated exposure.

This study aimed to assess immune status among individuals with chronic occupational exposure.

Materials and methods

The study was conducted in the Mayak Production Association (PA) workers cohort (Azizova et al. 2008). We included 91 workers in the study cohort who were first employed at one of the main facilities (reactors, radiochemical, plutonium production) in 1948–1972 as well as 43 individuals never employed at the Mayak PA (Table 1) followed up to 31.12.2008. Three groups of workers were formed to assess the immune status: (1) workers exposed to external gamma-rays at total dose 0.5–3.0 Gy (14 individuals); (2) workers with combined exposure (external gamma-rays of a total dose range 0.7–5.1 Gy) and internal alpha-radiation from incorporated plutonium with a body burden of 0.3–16.4 kBq (77 individuals); (3) the joint group of Mayak workers consisted of all individuals of the two groups mentioned above.

The control group (43 individuals) included individuals matched by age and sex, but never exposed occupationally, never involved in any cleanup operations following radiation accidents and never resided at contaminated areas. Characteristics of the study groups are given in Table 1.

The study was based on individual annual dose estimates using Mayak Workers Dosimetry System 2008 (MWDS-2008) developed in framework of Russian-American collaboration (Khohryakov et al. 2013). Individual annual doses from external gamma-rays were available for all Mayak workers. Plutonium body burden was measured only in 30 % of cohort members (Azizova et al. 2008); so, only those workers with measured plutonium body burden were involved in the study. Figure 1 demonstrates the distribution of Mayak workers in relation to the doses from external and internal exposure.

Of note, individuals with malignant neoplasms, acute inflammatory processes and exacerbation of chronic diseases registered at medical observation were excluded from the study.

Table 1 Characteristics of the study groups

Characteristics of the study groups	Mayak workers	Mayak workers with external gamma-rays	Mayak workers with combined exposure	Controls
Mean age of workers at the moment of biosampling, years (mean ± SE, median, range)	79.1 ± 0.55	78.2 ± 1.24	79.3 ± 0.60	77.4 ± 0.88
	79	77.5	79	77
	(66–91)	(69–85)	(66–91)	(63–92)
Mean age at exposure onset, years (mean ± SE, median, range)	21.4 ± 0. 36	22.4 ± 1.08	21.2 ± 0.38	
	21	22	20	
	(16–30)	(16–30)	(16–30)	
Mean duration of gamma-exposure, years (mean ± SE, median, range)	33.1 ± 1.41	38.6 ± 1.86	31.9 ± 1.61	
	36.0	38.5	34.5	
	(4–54)	(24–51)	(4–54)	
Total absorbed dose from external gamma-rays, Gy (mean ± SE, median, range)	1.76 ± 0.07	1.52 ± 0.21	1.82 ± 0.08	
	1.71	1.77	1.81	
	(0.47–5.09)	(0.47–2.92)	(0.73–5.09)	
Total absorbed red bone marrow (RBM) dose from internal alpha-radiation from incorporated ^{239}Pu, Gy (mean ± SE, median, range)	0.10 ± 0.01		0.10 ± 0.01	
	0.07		0.07	
	(0.01–1.01)		(0.01–1.01)	

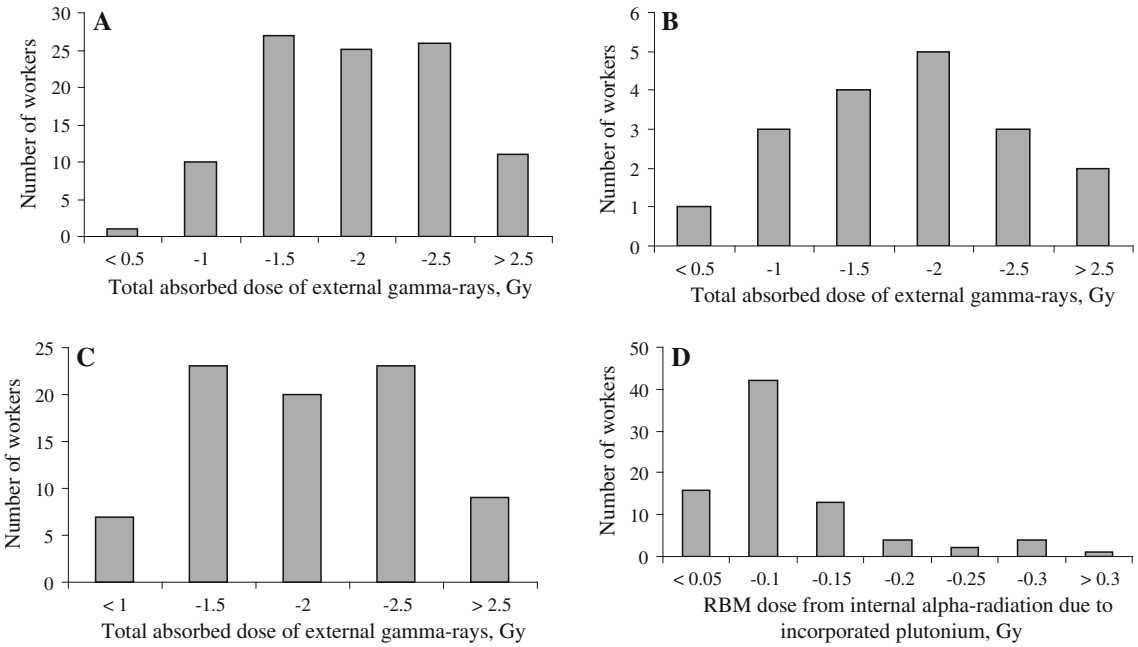

Fig. 1 Absorbed doses of Mayak workers. *Note*: **a** Distribution of all examined Mayak workers in relation to external gamma-ray dose. **b** Distribution of Mayak workers with external gamma-exposure in relation external gamma-ray dose. **c** Distribution of Mayak workers with combined exposure in relation to external gamma-ray dose; **d** Distribution of Mayak workers with combined exposure in relation to RBM dose from internal alpha-radiation due to incorporated plutonium

The project was endorsed by the SUBI Supervisory Board. All study participants signed the Informed Consent to Voluntary Participation in the Study and the Informed Consent to the Personal Data Treatment.

The study employed two methods: enzyme-linked immunoassay (ELISA) and flow cytometry (FCM). ELISA was used to analyze protein levels in peripheral blood serum including the cytokines: transforming growth factor

beta-1 (TGFβ1), tumor necrosis factor (TNFα), interferon gamma (IFNγ), interleukin-1 beta (IL-1β), interleukin-8 (IL-8), interleukin-10 (IL-10), immunoglobulins (IgM, IgG, IgA and IgE), transcription and apoptosis regulator p53, heat-shock protein-70 (Hsp70) and matrix metalloproteinase-9 (MMP-9).

Expression of lymphocyte membrane proteins (differentiation clusters) was determined by FCM according to relative and absolute concentrations of T-lymphocytes (CD3+CD19−), T-helpers (CD3+CD4+), cytoxic T-lymphocytes (CD3+ CD8+), B-lymphocytes (CD3−CD19+), natural killer (NK) cells (CD3−CD16+CD56+), natural killer T (NKT) cells (CD3+CD16+CD56+).

To perform ELISA, blood was drawn using 5-ml tubes (Sarstedt, Germany). Blood serum was extracted by centrifugation, portioned in 0.5-ml cryotubes (Sarstedt, Germany) and stored at −80 °C before use. To study protein levels in serum, we used sandwich ELISA according to the manufacturer's instructions of test systems, such as Bender Medsystems (Austria), Biosourse (USA), Seramun (Germany), Monobind (USA) and BD Biosciences (USA). Results were interpreted using microplate photometer for ELISA Stat Fax 2100 (Awareness Technology, Inc, USA).

For flow cytometry (FCM) analysis, blood was drawn from all study participants into 5-ml tubes with lithium heparin (Sarstedt, Germany). Lymphocytes differentiation clusters were examined by flow cytometer Fc500 (Beckman Coulter, USA) and an automatic Q-PREP workstation (Beckman Coulter, USA).

The FCM analysis was based on direct immune-fluorescence in whole peripheral blood and washless technique (Beckman Coulter, USA).

Statistical analysis of results was based on Spearman's rank correlation coefficient and linear regression. Statistical significance of regression parameters was estimated using Student's t test, and significance of regression was estimated by Fisher's F test. Analysis of the distribution pattern of investigated parameters showed that values do not obey normal distribution. Null hypothesis was analyzed by nonparametric Mann–Whitney test. A p value of <0.05 was assumed to be statistically significant.

Results

Results of comparison of study parameters between Mayak workers with chronic exposure and controls are given in the "Appendix."

Statistically significant differences between parameters within the study groups are presented in Figs. 2 and 4. Relative concentrations of T-lymphocytes were decreased in exposed Mayak workers of all study groups as compared with controls ($p < 0.05$) (Fig. 2a).

Absolute concentrations of T-lymphocytes were not significant different in workers with chronic exposure as compared to control ("Appendix"). However, this parameter significantly decreased with increasing total absorbed dose from external gamma-rays (Table 2).

Relative concentrations of T-helpers were not significant different between workers with only chronic gamma-exposure and the control group ("Appendix"), and this parameter was found to be related to total absorbed dose from external gamma-rays. The relation between dose and T-helper counts was linear and inverse, both in workers with only gamma-exposure ($r = -0.66$, $p = 0.01$; Fig. 3) and in all workers with chronic exposure (Table 2). In addition, absolute concentrations of T-helpers in all groups of Mayak workers showed no significant differences as compared to controls ("Appendix"). However, the concentrations decreased ($p < 0.05$) with increasing either total absorbed dose from external gamma-rays or total absorbed red bone marrow (RBM) dose from internal alpha-radiation (Table 2). Relative concentration of NKT-lymphocytes in workers with external exposure significantly increased as compared with controls (Fig. 2b; "Appendix"). Relative concentration of NKT-lymphocytes increased with increasing total absorbed RBM dose from internal alpha-radiation (Table 2).

Relative and absolute concentrations of natural killer cells were increased relative to control ($p < 0.05$) in Mayak workers with chronic exposure as well as in workers with combined exposures (Fig. 2c, d).

For B-lymphocytes and T-cytotoxic lymphocytes, the relative and absolute concentrations were not different between controls and Mayak workers, irrespective whether the latter had been exposed to external gamma-rays nor internal alpha-radiation ("Appendix").

For the cytokines, the concentration of TGFβ was increased ($p < 0.05$) in all Mayak workers as well as in workers with external gamma-exposure as compared with controls (Fig. 4a). There was a significant decrease of IL-8 concentration ($p < 0.05$) in all groups of Mayak workers as compared with controls (Fig. 4b). For IFNγ, there was an increase in workers with external gamma-exposure ($p < 0.05$; Fig. 4c) relative to control, while there was an inversely correlated decrease ($p < 0.05$) with the total absorbed dose from external gamma-exposure (Table 2). Concentrations of immunoglobulins A and M and MMP-9 were increased in all groups of Mayak workers as compared with control (Fig. 4d–f).

Concentrations of IL-1β, IL-6, IL-10, TNFα, VEGF, IgE, p53 and Hsp 70 in blood serum were similar in workers with occupational exposure and controls ("Appendix").

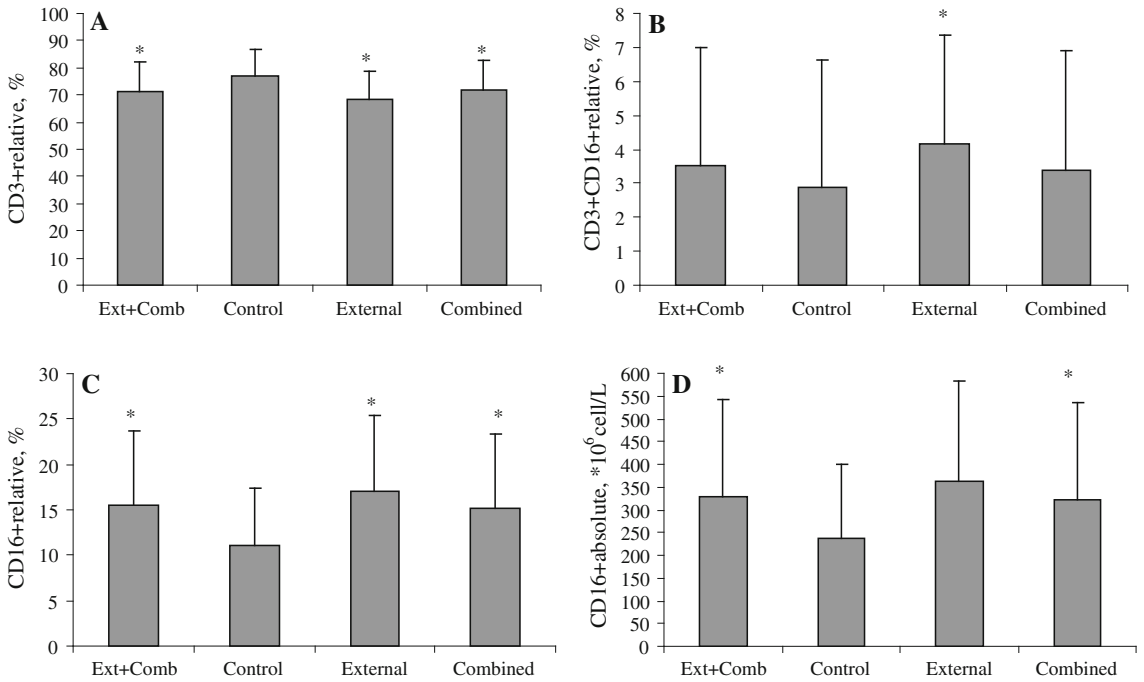

Fig. 2 Concentration of different lymphocyte subsets in workers with chronic exposure. *Note*: **a** Relative concentration of T-lymphocytes, **b** relative concentration of NKT-lymphocytes, **c** relative concentration of NK-cells, **d** absolute concentration of NK-cells

Table 2 Parameters of regression equations

Parameter	*N*	*r*	*p*	*a*	*b*
Linear relations of study parameters to total absorbed dose from external gamma-rays (Gy)					
T-lymphocytes (absolute concentration, 10^6 cell/l)	91	−0.26	0.049	1,920.40 ± 77.63	246.16 ± 97.15
T-helpers (relative concentration, %)	91	−0.22	0.04	46.81 ± 2.63	3.07 ± 1.44
T-helpers (absolute concentration, 10^6 cell/l)	91	−0.29	0.0056	1,095.61 ± 88.76	138.01 ± 48.55
IFN (pg/ml)	91	−0.28	0.007	2.19 ± 0.48	0.73 ± 0.26
Linear relations of study parameters to total absorbed RBM dose from internal alpha-radiation (Gy)					
T-helpers (absolute concentration, 10^6 cell/l)	77	−0.27	0.02	991.55 ± 58.96	1,333.70 ± 562.88
NKT-lymphocytes (relative concentration, %)	77	0.27	0.02	1.95 ± 0.73	16.80 ± 6.97

N number of examined individuals, *r* correlation rate, *p* significance level of regression equation, *a* intercept of regression equation, *b* slope coefficient

Discussion

Lymphocytes are one of the most radiosensitive cells (Anderson and Warner 1976; Hendry 1988; Eidus et al. 1990), while their radiosensitivity varies between different subsets (Kwan and Norman 1977). This study showed that absolute concentrations of T-lymphocytes in a cohort of previously occupationally exposed Mayak workers decreased with increasing total absorbed dose from external gamma-rays, even years after the initial exposures. Absolute concentrations of T-helpers decreased with increasing dose both from external and internal exposure of the workers. The results obtained in this study are in accordance with data from the analogous study in workers of reactors and plutonium production facilities of Seversk Chemical Combine (nuclear enterprise similar to Mayak PA; Tahauov et al. 2003, 2005; Voronova 2007), as well as in Hiroshima and Nagasaki A-bomb survivors (Akiyama 1995). The dose-dependant decrease in concentration of T-lymphocytes and T-helpers, especially years after external gamma-exposure, has not been explained yet. In workers with combined exposures, lymphocytes and their stem cells are still continuously exposed to internal alpha-radiation after nuclide incorporation and, probably, ongoing cell death depletes their numbers. For external gamma-rays, the chronic exposure stopped several decades ago still

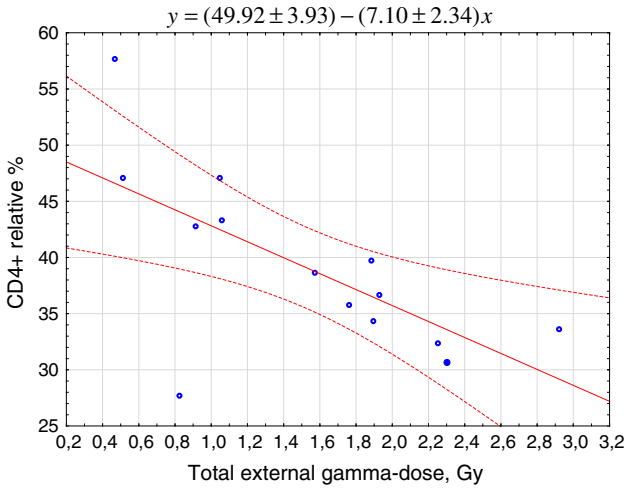

Fig. 3 Relative concentration of T-helpers in relation to the total absorbed dose from external gamma-rays in Mayak workers with external gamma radiation only. *Note*: *Y* axis: the relative concentration of T-helpers in percent, *X* axis: the total absorbed dose from external gamma-rays in Gy

leaving an imprint on the lymphocyte subtype composition. It can be assumed that the depletion of T-lymphocyte precursor cells in Mayak workers during their early adulthood (≥21a).

Recovery of a depleted T-lymphocyte pool from stem cells among exposed individuals could not fully occur as their thymus already functioned poorly due to its age-related involution. It has been known that T-lymphocytes mature in thymus which starts degrading soon after birth (Kusunoki et al. 1998). Decreased numbers of CD4+ lymphocytes were also observed among Hiroshima and Nagasaki A-bomb survivors 42–46 years after acute exposure (Kusunoki et al. 1998, 1999), in residents of Techa riverside villages 53–56 years after exposure onset (Ovcharova 2006; Akleyev 2007); relation of CD4+ lymphocytes to dose was revealed both in this study and the Japanese one (Kusunoki et al. 1998) and in gamma radiation-exposed individuals of the Lilo accident (Scherthan et al. 2007). Relative and absolute concentration of CD8+ subset of T-lymphocytes was altered neither in this study nor in Japanese cohort (Kusunoki et al. 1998). No alterations in concentration of B-lymphocytes were found in Hiroshima and Nagasaki A-bomb survivors; this study revealed increased concentration of immunoglobulins A and M consistent with the Japanese data (Akiyama 1995).

Increased relative concentrations of NKT cells were found in workers with external gamma-exposure. However, no difference between workers with combined exposure and control was found, but there was a significant increase with increasing total RBM dose from internal alpha-radiation, which is in agreement with the studies on individuals with combined exposure (Kirillova et al. 2012).

Increase in concentrations of cytokines and immunoglobulins in the delayed period after occupational chronic radiation exposure remains unexplained. Proinflammatory cytokines can be activated soon after tissue exposure. Ionizing radiation produces free reactive oxygen and nitrogen species, which may activate and stimulate the production of proinflammatory cytokines that themselves generate reactive oxygen species. As a result, this chain of interactions sustains tissue inflammation, able to persist for a long time (Schaue et al. 2012). Japanese studies on immune and cytokine status in Hiroshima and Nagasaki A-bomb survivors revealed that increased expression of proinflammatory cytokines in the delayed period after exposure was associated with a decreased concentration of Tn subsets of T-lymphocytes in blood (Kusunoki et al. 2010). Another explanation is that an immune system affected by radiation is less able to control microbial infections, which in turn can cause chronic inflammation and non-cancer diseases that are fostered due to unregulated and permanent inflammation. Radiation-induced genome instability or bystander effect is also assumed to initiate inflammatory response, which can last for years (Hayashi et al. 2003, 2005, 2012; Lorimore et al. 2003; Neriishi et al. 2001). In this study, the increased concentrations of proinflammatory cytokines IFNγ and immunoglobulins M and A in exposed workers indicated enhancement of inflammatory response, both in terms of external and combined exposure, while there also was a decrease in the concentration of proinflammatory interleukin-8 and increase in concentration of cytokine TGFβ in the worker's blood serum.

\ This study also shows that the concentration of interferon gamma increased in exposed workers due to radiation exposure, which is consistent with studies in A-bomb survivors (Hayashi et al. 2003).

The concentration of TGFβ increases after radiation therapy and its increase can persist (Dancea et al. 2009), which is consistent with the results from the current study.

It has been known that different subsets of lymphocytes together with cytokines may play critical role in pathogenesis of many diseases. In particular, higher incidence of myocardial infarction was revealed in Hiroshima and Nagasaki A-bomb survivors with decreased concentration of CD4+ lymphocytes as compared with those with increased CD4+ concentration (Kusunoki et al. 1999).

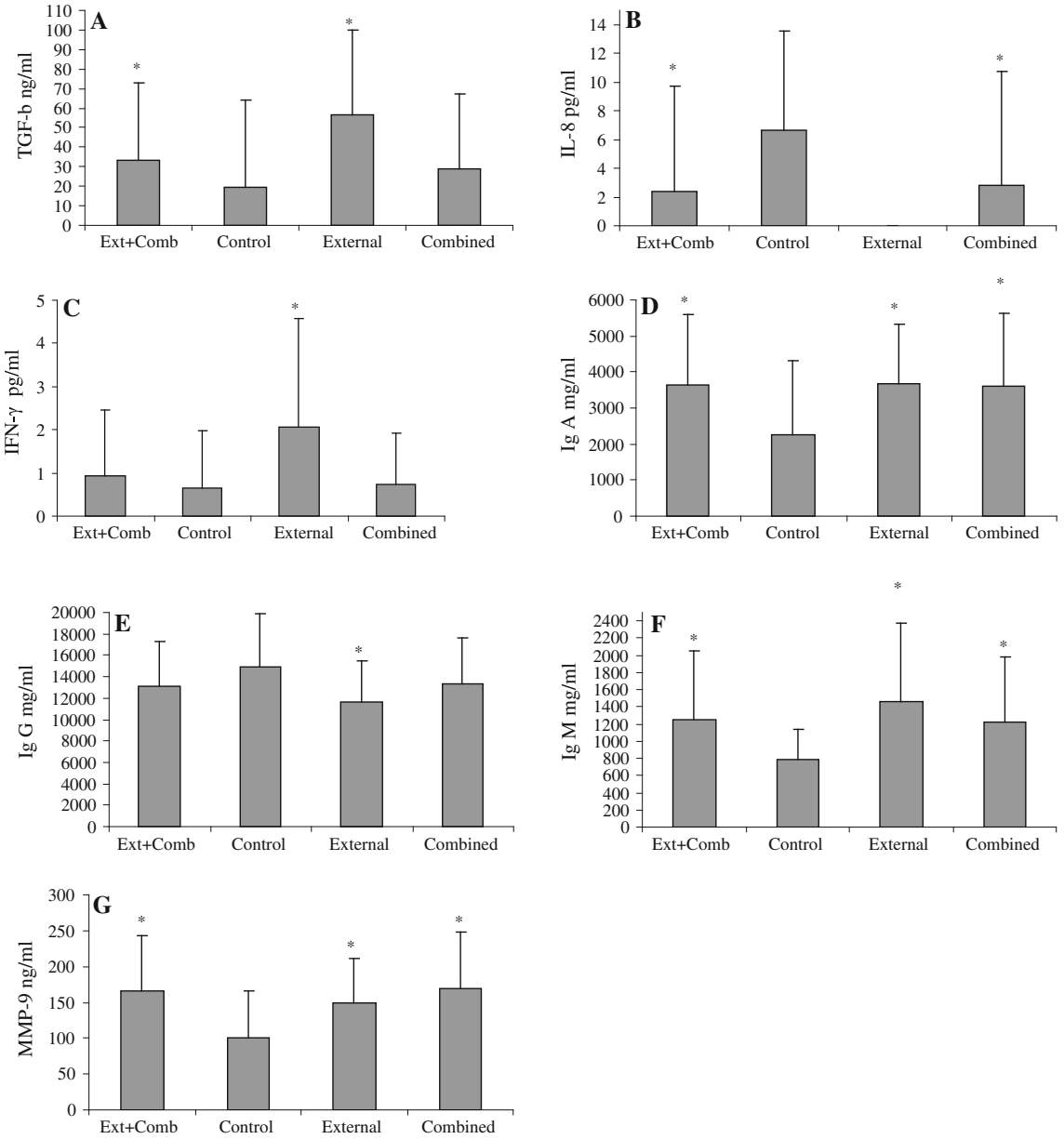

Fig. 4 Concentration of blood serum proteins among individuals with chronic exposure. *Note*: **a** Concentration of TGF β, **b** concentration of, IL-8, **c** concentration of IFNγ, **d** concentration of IgA, **e** concentration of IgG, **f** concentration of IgM, **g** concentration of MMP-9

TGFβ effects on the immune system are predominantly of inhibitory nature. At the same time, it is important for humoral response as it switches immunoglobulins biosynthesis to IgA-isotype, which, probably, explains increased IgA synthesis in exposed individuals. Some cytokines, such as TGFβ1 and IL-1 beta, can stimulate radiation-induced proliferation of endothelium, fibroblasts, collagen deposition and fibrosis leading to increase in arteriosclerosis area (Basavaraju and Easterly 2002; Border and Noble 1994; Grainger 2007).

In terms of external gamma-exposure in Mayak workers, absolute and relative concentrations of NKT cells were increased in those with external gamma-rays only, while there was no significant difference between workers with combined exposure and control. However, NKT concentrations increased with increasing total RBM dose from

internal alpha-radiation. NKT cells are heterogeneous subset of specific autoreactive T cells with innate and adaptive immune features allowing combination of innate and adaptive immunity to prevent from autoimmune and malignant diseases. NKT cells can rapidly express pro- and anti-inflammatory cytokines determining the type and the magnitude of the immune response (Subleski et al. 2006; Klucinski et al. 2011).

Within the immune system, cytoxic T-lymphocytes and T-helper type 1 cells together with their interferon gamma act as antitumor effectors. Interferon gamma is produced by T cells and natural killers in response to allogenic antigens or mitogens, as well as other factors including radiation exposure. In addition to decreased concentration of T-lymphocytes and T-helpers, this study revealed increased interferon gamma synthesis probably due to increased concentration of natural killers, which may also be due to interferon effects. Hence, in spite of the reduction of T-lymphocytes and T-helpers concentrations, this effect on the antitumor defense can be compensated to some extent by an increased concentration of natural killers and interferon. While TGFβ plays an ambiguous role in tumor development and survival, an increased concentration of TGFβ revealed in the current study may have the potential to promote fibrosis (Biswas et al. 2007; Border and Noble 1994).

The matrix metalloproteinase MMP-9 can degrade extracellular matrix proteins and plays a role in tumor invasion (e.g., Merdad et al. 2014). MMP-9 degrades denatured collagen and collagen type 4, a major component of basal membrane. This function facilitates lymphocytes and other leukocytes entering and exiting the blood and lymphatic channels as well as their contribution to inflammatory process. In most cell types, transcription of MMP-9 gene is induced by cytokines such as IFNγ the concentration of which was increased in Mayak workers; thus, MMP-9 increased transcription reveled in this study may relate to increased inflammatory processes in the RBM of the exposed individuals. In addition, TGFβ suppresses the function of metalloproteinase inhibitors, which also may lead to increased concentration of MMP-9 in blood serum. Metalloproteinases play a role in pathological inflammatory processes including arthritis, cardiovascular diseases, pulmonary diseases and cancer (Ram et al. 2006).

Conclusions

Our current study of serum protein expression and cell composition of the peripheral blood of aged and previously chronically radiation-exposed Mayak workers showed that the main alterations were related to T-components of the immune system and associated with dose-dependent decrease of the concentrations of T-lymphocytes and T-helpers as well as increased tumor-supporting protein concentrations (IgM, IgA and MMP-9).

An inverse linear relationship of absolute concentration of T-lymphocytes, relative and absolute concentrations of T-helpers, concentration of interferon gamma to total absorbed dose from external gamma-rays were revealed in exposed individuals. Inverse linear relation of absolute concentration of T-helpers and direct linear relation of relative concentration of NKT-lymphocytes to total absorbed RBM dose from internal alpha-radiation due to incorporated plutonium were established.

It should be noted that relations established in this study between immune system components and total absorbed doses from external gamma-rays and internal alpha-radiation due to incorporated plutonium were small but statistically significant; group changes in these components did not exceed control ones.

Alterations in T and B components of immune system in cytokine status revealed in this study may sustain chronic inflammation and partially contribute to cardiovascular, malignant and other diseases (Kusunoki et al. 2010; Packard et al. 2009; Zamarron and Chen 2011). The study on changes in expression of the immune system proteins allows a better understanding of the mechanisms of late radiation effects. In the future, we plan to refine the data obtained by increasing the statistical power of the study.

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Appendix

See Table 3.

Table 3 Concentration of lymphocytes differentiation clusters and blood serum proteins among individuals with external and combined exposure

Parameters	Mayak workers ($N = 91$)		Mayak workers with external gamma-exposure ($N = 14$)		Mayak workers with combined exposure ($N = 77$)		Controls ($N = 43$)	
	Mean ± SE SD	Median (Min–Max)	Mean ± SE SD	Median (Min–Max)	Mean ± SE SD	Median (Min–Max)	Mean ± SE SD	Median (Min–Max)
T-lymphocytes percentage (%)	71.2 ± 1.1[a] 10.8	71.8 (42.9–89.7)	68.2 ± 2.8[a] 10.6	69.8 (46.2–80.9)	71.8 ± 2.8[a] 10.8	72.2 (42.9–89.7)	76.9 ± 1.5 10.0	79.3 (50.8–92.3)
T-lymphocytes absolute (10^6 cell/l)	1,492 ± 58 564	1,370 (542–3,628)	1,414 ± 137 515	1,390 (542–2,199)	1,505 ± 137 575	1,370 (681–3,628)	1,653 ± 88 574.6	1,644 (669–3,572)
T-helpers percentage (%)	41.3 ± 0.9 8.4	41.6 (21.9–60.2)	39.1 ± 2.1 7.9	37.6 (27.7–57.6)	41.7 ± 2.1 8.4	42.4 (21.9–60.2)	40.0 ± 1.7 11.0	41.9 (17.5–60.9)
T-helpers absolute (10^6 cell/l)	853 ± 30 285	836 (403–1,823)	791 ± 70 265	826 (449–1,294)	864 ± 70 288	836 (403–1,823)	849 ± 55.2 357.8	780 (277–2,209)
T-cytoxic lymphocytes percentage (%)	26.8 ± 1.0 9.9	25.8 (8.1–55.1)	26.8 ± 2.4 9.1	27.8 (12.6–39.1)	26.8 ± 2.4 10.2	25.8 (8.1–55.0)	28.1 ± 1.7 11.3	25.8 (10.2–57.6)
T-cytoxic lymphocytes absolute (10^6 cell/l)	568 ± 32 310	514 (100–1,925)	567 ± 73.2 274	548 (100–1,077)	568 ± 73 317	508 (166–1,925)	612 ± 50 324.3	586 (186–1,357)
NKT-lymphocytes percentage (%)	3.5 ± 0.3 3	2.6 (0.2–15.4)	4.2 ± 0.8[a] 3.2	3.5 (0.3–11.7)	3.4 ± 0.8 3.5	2.5 (0.2–15.4)	2.9 ± 0.5 3.7	1.7 (0.2–16.0)
NKT-lymphocytes absolute (10^6 cell/l)	72 ± 7 70	54 (4–373)	88 ± 19[a] 72	67 (5–263)	68 ± 19 69	51 (4–373)	66 ± 15 97.7	31 (5–432)
NK-lymphocytes percentage (%)	15.4 ± 0.9[a] 8.8	13.8 (2.4–39.4)	17.1 ± 2.2[a] 8.3	15.4 (4.9–39.4)	15.1 ± 2.2[a] 8.3	13.4 (2.4–38.3)	11.2 ± 0.9 6.2	9.3 (3.5–29.6)
NK-lymphocytes absolute (10^6 cell/l)	328 ± 22[a] 215	282 (35–1,054)	364 ± 58 219	300 (107–676)	321 ± 589[a] 215	282 (35–1,054)	238 ± 25 162.2	189 (55–759)
B-lymphocytes percentage (%)	8.3 ± 0.4 3.8	7.9 (2.4–26.0)	8.3 ± 0.6 2.4	8.2 (4.4–12.8)	8.3 ± 0.7 3.9	7.7 (2.4–26.0)	8.0 ± 0.5 3.6	7.4 (1.2–18.7)
B-lymphocytes absolute (10^6 cell/l)	176 ± 10 101	154 (29–500)	173.5 ± 20.4 76	198 (58–288)	176 ± 20 105	140 (29–500)	169 ± 13 87.4	155 (30–470)
IL-1b (pg/ml)	0.1 ± 0.05 0.5	0.0 (0.0–3.2)	0.5 ± 0.2[a] 0.9	0.0 (0.0–2.4)	0.1 ± 0.2 0.4	0.0 (0.0–3.20)	0.5 ± 0.5 3.2	0.0 (0.0–21.2)
IL-6 (pg/ml)	3.2 ± 0.5 4.4	1.8 (0.0–28.8)	1.6 ± 0.6 2.3	0.7 (0.0–8.6)	3.5 ± 0.6 4.6	2.2 (0.0–28.8)	3.5 ± 1.0 6.8	1.3 (0.0–36.6)
IL-8 (pg/ml)	2.4 ± 0.8[a] 7.3	0.0 (0.0–56.0)	0.0 ± 0.0[a,b] 0.0	0.0 (0.0–0.0)	2.8 ± 0.0[a] 7.9	0.0 (0.0–56.00)	6.6 ± 1.0 6.9	5.2 (0.0–26.2)
IL-10 (pg/ml)	3.0 ± 1.2 11.5	0.4 (0.0–87.0)	7.2 ± 4.8 17.8	0.1 (0.0–64.9)	2.3 ± 4.7 9.9	0.4 (0.0–87.0)	13.3 ± 12.5 81.5	0.1 (0.0–529.0)

Table 3 continued

Parameters	Mayak workers ($N = 91$)		Mayak workers with external gamma-exposure ($N = 14$)		Mayak workers with combined exposure ($N = 77$)		Controls ($N = 43$)	
	Mean ± SE SD	Median (Min–Max)	Mean ± SE SD	Median (Min–Max)	Mean ± SE SD	Median (Min–Max)	Mean ± SE SD	Median (Min–Max)
TGF-β (ng/ml)	33.4 ± 4.1[a] 39.8	21.6 (0.0–174.1)	56.7 ± 11.5[a,b] 43.1	55.8 (0.0–142.3)	29.2 ± 11.5 37.9	10.5 (0.0–174.1)	19.5 ± 6.8 44.6	7.0 (0.0–210.9)
TNF-α (pg/ml)	0.2 ± 0.3 1.3	0.0 (0.0–9.2)	0.0 ± 0.0 0.0	0.0 (0.0–0.0)	0.3 ± 0.0 1.4	0.0 (0.0–9.2)	0.0 ± 0.0 0.0	0.0 (0.0–0.0)
IFNγ (pg/ml)	0.9 ± 0.2 1.5	0.3 (0.0–8.6)	2.1 ± 0.7[a,b] 2.5	1.3 (0.0–8.6)	0.7 ± 0.7 1.2	0.1 (0.0–7.8)	0.6 ± 0.2 1.3	0.0 (0.0–6.9)
VEGF (pg/ml)	351.0 ± 36.5 349.8	236.7 (0.0–1,626.0)	297.5 ± 97.8 365.9	167.1 (30.4–438.8)	360.6 ± 97.8 348.4	249.0 (0.0–1,626.0)	427.1 ± 55.8 362.162	389.3 (7.2–1,850.0)
Ig A (mg/ml)	3.6 ± 0.20[a] 1.9	3.0 (0.8–11.0)	3.7 ± 0.4[a] 1.6	3.1 (1.0–6.5)	3.6 ± 0.4[a] 2.0	3.0 (0.78–11.4)	2.3 ± 0.3 2.0	1.2 (0.2–8.6)
Ig E (IU/ml)	91.3 ± 11.7 112.3	53.0 (2.3–636.1)	101.5 ± 28.3 105.8	61.6 (5.4–362.9)	89.4 ± 28.3 114.0	50.05 (2.3–636.1)	95.5 ± 21.8 141.7	47.2 (5.5–609.5)
Ig G (mg/ml)	13.0 ± 0.4 4.2	12.1 (6.9–32.0)	11.6 ± 1.0[a] 3.8	10.7 (7.0–22.4)	13.3 ± 1.0 4.2	1.7 (6.9 –32.0)	14.9 ± 0.8 5.0	13.2 (8.4–24.7)
Ig M (mg/ml)	1.2 ± 0.08[a] 0.8	1.0 (0.3–4.0)	1.4 ± 0.2[a] 0.9	1.2 (0.4–3.6)	1.2 ± 0.2[a] 767.5	1.0 (0.3–4.0)	0.8 ± 0.05 0.4	0.6 (0.3–2.1)
p53 (U/ml)	0.4 ± 0.08 0.8	0.2 (0.0–6.8)	1.0 ± 0.5[b] 1.7	0.5 (0.0–6.8)	0.4 ± 0.5 0.5	0.1 (0.0–2.4)	1.2 ± 0.8 5.1	0.1 (0.0–33.6)
Hsp 70 (ng/ml)	0.68 ± 0.2 2.2	0.0 (0.0–15.5)	0.5 ± 0.2 0.8	0.1 (0.0–2.6)	0.7 ± 0.2 2.4	0.0 (0.0–15.5)	2.6 ± 1.3 8.1	0.0 (0.0–34.8)
MMP-9 (ng/ml)	166.1 ± 8.0[a] 77.1	156.4 (23.0–467.0)	149.7 ± 16.3[a] 60.9	147.1 (76.3–277.5)	169.0 ± 16.3[a] 79.6	157.1 (23.0–467.0)	99.8 ± 10.2 66.0	66.5 (20.0–280.0)

[a] The value significantly differs from the corresponding value in control

[b] The value statistically significantly differs from corresponding value in Mayak workers with combined exposure

References

Akiyama M (1995) Late effects of radiation on the human immune system: an overview of immune response among the atomic-bomb survivors. Int J Radiat Biol 68:497–508

Akleyev AV (2007) Long-term immunity status of individuals exposed to chronic radiation. Med Radiol Radiat Saf 52:5–9

Akleyev AV (2012) Chronic radiation syndrome in Techa riverside residents. Book, Chelyabinsk

Anderson RE, Warner NL (1976) Ionizing radiation and the immune response. Adv Iommunol 24:215–235

Azizova TV, Day RD, Wald N, Muirhead CR, OHagan JA, Sumina MV, Belyaeva ZD, Druzhinina MB, Teplyakov II, Seminikhina NG, Stetsenko LA, Grigoryeva ES, Krupenina LN, Vlasenko EV (2008) The "Clinic" medical-dosimetric database of Mayak production association workers: structure, characteristics and prospects of utilization. Health Phys 94:449–458

Basavaraju SR, Easterly CE (2002) Pathophysiological effects of radiation on atherosclerosis development and progression and the incidence of cardiovascular complications. Med Phys 29:2391–2403

Biswas S, Guix M, Rinehart C, Dugger TC, Chytil A, Moses HL, Freeman ML, Arteaga CL (2007) Inhibition of TGF-β with neutralizing antibodies prevents radiation-induced acceleration of metastatic cancer progression. J Clin Invest 117:1305–1313. doi:10.1172/JCI30740

Border WA, Noble NA (1994) Transforming growth factor *b* in tissue fibrosis. N Engl J Med 331:1286–1292

Courtade M (2001) Influence of continuous very low-dose gamma-irradiation on the mouse immune system. Int J Radiat Biol 77:587–592

Dancea HC, Shareef MM, Ahmed MM (2009) Role of radiation-induced TGF-beta signaling in cancer therapy. Mol Cell Pharmacol 1:44–56

Duhrsen U, Metcalf D (1990) Effects of irradiation of recipient mice on the behavior and leukemogenic potential of factor-dependent hematopoietic cell lines. Blood 75:190–197

Eidus LK et al (1990) The mechanism of radiation induced interphase death of lymphoid cells: a new hypothesis. Radiat Res 123:17–21

Grainger DJ (2007) TGF-b and atherosclerosis in man. Cardiovasc Res 74:213–222

Greenberger JS, Epperly MW, Zeevi A et al (1996) Stromal cell involvement in leukemogenesis and carcinogenesis. In Vivo 10:1–17

Hayashi T, Kusunoki Y, Hakoda M, Kubo Y, Morishita Y, Maki M, Kasagi F, Kodama K, Macphee DG, Kyoizumi S (2003) Radiation dose-dependent increases in inflammatory response markers in A-bomb survivors. Int J Radiat Biol 79:129–136

Hayashi T, Morishita Y, Kubo Y, Kusunoki Y, Hayashi I, Kasagi F, Hakoda M, Kyoizumi S, Nakachi K (2005) Long-term effects of radiation dose on inflammatory markers in atomic bomb survivors. Am J Med 118:83–86

Hayashi T, Morishita Y, Khattree R, Misumi M, Sasaki K, Hayashi I, Yoshida K, Kajimura J, Kyoizumi S, Imai K, Kusunoki Y, Nakachi K (2012) Evaluation of systemic markers of inflammation in atomic-bomb survivors with special reference to radiation and age effects. FASEB J 26. www.fasebj.org (The FASEB Journal article fj.12-215228. Published online August 7, 2012)

Hendry JH (1988) Survival of cells in mammalian tissues after low doses of irradiation: a short review. Int J Radiat Biol Relat Stud Phys Chem Med 53:89–94

Ishioka N, Umeki S, Hirai Y et al (1997) Stimulated rapid expression in vitro for early detection of in vivo T-cell receptor mutations induced by radiation exposure. Mutat Res 390:269–282

Khohryakov VV, Khohryakov VF, Suslova KG, Vostrotin VV, Vvedensky VE, Sokolova AB, Krahenbuhl MP, Birchall A, Miller SC, Schadilov AE, Ephimov AV (2013) Mayak Worker Dosimetry System 2008 (MWDS-2008) Assessment of internal alpha-dose from measurement results of plutonium activity in urine. Health Phys 104:366–378

Kirillova EN, Muksinova KN, Skukovskaia TL (1988) Effect of prolonged continuous external irradiation on humoral immunity indices of mice. Kosm Biol Aviakosm Med 22:62–65

Kirillova EN, Zakharova ML, Muksinova KN, Drugova ED, Pavlova OS, Sokolova SN (2012) Quantitative assessment of regulatory proteins in blood as markers of radiation affects in the late period after occupational exposure. Health Phys 103:28–36

Kłuciński P, Subleski JJ, Jiang Q, Weiss JM, Wiltrout RH (2011) The split personality of NKT cells in malignancy autoimmune and allergic disorders. Immunotherapy 3:1167–1184

Kolesnik R, Fux Z (2003) Radiation and ceramide-induced apoptosis. Oncogene 22:5897–5906

Kusunoki Y, Kyoizumi S, Hirai Y, Suzuki T, Nakashima E, Kodama K, Seyama T (1998) Flow cytometry measurements of subsets of T, B and NK cells in peripheral blood lymphocytes of atomic bomb survivors. Radiat Res 150:227–236

Kusunoki Y, Kyoizumi S, Yamaoka M, Kasagi F, Kodama K, Seyama T (1999) Decreased proportion of CD4 T cells in the blood of atomic bomb survivors with myocardial infarction. Radiat Res 152:539–543

Kusunoki Y, Yamaoka M, Kasagi F et al (2003) 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 122:975–984

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

Kusunoki Y, Yamaoka M, Kubo Y, Hayashi T, Kasagi F, Douple EB, Nakachi K (2010) T-cell immunosenescence and inflammatory response in atomic bomb survivors. Radiat Res 174:870–876

Kwan DK, Norman A (1977) Radiosensitivity of human lymphocytes and thymocytes. Radiat Res 69:143–151

Kyoizumi S, Umeki S, Akiama M et al (1992) Frequency of mutant T-lymphocytes defective in the expression of the T-cell antigen receptor gene among radiation-exposed people. Mutat Res 265:173–180

Liao YP, Wang CC, Butterfield LH et al (2004) Ionizing radiation affects human MART-1 melanoma antigen processing and presentation by dendritic cells. J Immunol 173(4):2462–2469

Liu XD, Ma SM, Liu SZ (2003) Effects of 0.075 Gy x-ray irradiation on the expression of IL-10 and IL-12 in mice. Phys Med Biol 48:2041–2049

Lorimore SA, Coats PJ, Wright EG (2003) Radiation-induced genomic instability and bystander effects: inter-related nontargeted effects of exposure to ionizing radiation. Oncogene 22:7058–7069

Merdad A, Karim S, Schulten HJ, Dallol A, Buhmeida A, Al-Thubaity F, Gari MA, Chaudhary AG, Abuzenadah AM, Al-Qahtani MH (2014) Expression of matrix metalloproteinases (MMPs) in primary human breast cancer: MMP-9 as a potential biomarker for cancer invasion and metastasis. Anticancer Res 34:1355–1366

Nakachi K, Hayashi T, Imai K et al (2004) Perspectives of cancer immuno-epidemiology. Cancer Sci 95:921–929

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

Nikolic B, Lee S, Bronson RT et al (2000) Th1 and Th2 mediate acute graft-versus host disease each with distinct end-organ targets. J Clin Invest 105:1289–1298

Oldstone MB (1998) Molecular mimicry and immune-mediated diseases. FASEBJ 12(13):1255–1265

Ovcharova EA (2006) Long-term effects of low dose rate chronic radiation exposure on immunity parameters in Techa riverside village residents. Dissertation, Moscow State University

Packard RRS, Lichtman AH, Libby P (2009) Innate and adaptive immunity in atherosclerosis. Semin Immunopathol 31:5–22. doi:10.1007/s00281-009-0153-8

Pandey R, Shankar BS, Sharma D, Sainis KB (2005) Low dose radiation induced immunomodulation: effect on macrophages and CD8+ T cells. Int J Radiat Biol 81:801–812

Ram M, Sherer Y, Shoenfeld Y (2006) Matrix metalloproteinase-9 and autoimmune diseases. J Clin Immunol. doi:10.1007/s10875-006-9022-6

Rees GS, Daniel CP, Morris SD, Whitehouse CA, Binks K, MacGregor DH, Tawn EJ (2004) Occupational exposure to ionizing radiation has no effect on T- and B-total counts or percentages of helper, cytotoxic and activated T-cell subsets in the peripheral circulation of male radiation workers. Int J Radiat Biol 180:493–498

Schaue D, Kachikwu EL, McBride WH (2012) Cytokines in radiobiological responses: a review. Radiat Res 178:505–523

Scherthan H, Abend M, Müller K, Beinke C, Braselmann H, Zitzelsberger H, Köhn FM, Pillekamp H, Schiener R, Das O, Peter RU, Herzog G, Tzschach A, Dörr HD, Fliedner TM, Meineke V (2007) Radiation-induced late effects in two affected individuals of the Lilo radiation accident. Radiat Res 167:615–623

Shankar B, Sainis KB (2005) Cell cycle regulators modulating con A mitogenesis and apoptosis in low-dose radiation-exposed mice. J Environ Pathol Toxicol Oncol 24:33–43

Subleski JJ, Hall VL, Back TC, Ortaldo JR, Wiltrout RH (2006) Enhanced antitumor response by divergent modulation of natural killer and natural killer T cells in the liver. Cancer Res 66:11005–11012

Tahauov RM, Sherstoboev EU, Voronova IA, Uduy VV, Carpov AB, Semyonova UV, Churin AA, Koltunov AA, Borodulina EV, Gribov SA, Avdeeva OV (2003) Complex assessment of unspecific resistance and antioxidant defense alterations and their amendment in workers of radiation hazardous production facilities. Sib Med Mag 18:64–71

Tahauov RM, Carpov AB, Udut VV, Semyonova UV, Sherstoboev EU, Borodulina EV, Blokhina TV, Marsheva SI, Babakina NV (2005) Assessment of homeostatic balance in workers of radiation hazardous production facilities exposed to external gamma-rays. Thesis report for the Third International Workshop Chronic radiation exposure: medical and biological effects: Chelyabinsk State Medical Academy, Chelyabinsk

Umeki SY, Suzuki Y, Kusunoki JB et al (1997) Development of a mouse model for studying in vivo T-cell receptor mutations. Mutat Res 393:37–46

United Nations (2006) Effects of ionizing radiation. Volume II: Scientific Annex D. United Nations Scientific Committee on the Effects of Atomic Radiation, UNSCEAR 2006 Report. United Nations, New York

Voronova IA (2007) Some peculiarities of immune status changes in workers of radiation hazardous production facilities exposed to low doses of ionizing radiation. "Medical and ecological effects of ionizing radiation (MEEIR-IV"): deliberations of the forth international research and practice conference dedicated to the 50th anniversary of Branch2 of the State Research Center—Biophysics Institute—Seversk—Tomsk

Westermann W, Schobl R, Rieber EP et al (1999) Th2 cells as effectors in postirradiation pulmonary damage preceding fibrosis in the rat. Int J Radiat Biol 75:629–638

Yamaoka M, Kusunoki Y, Kasagi F et al (2004) Decreases in percentages of naive CD4 and CD8 T-cells and increases in percentages of memory CD8 T cell subsets in the peripheral blood lymphocyte populations of atomic bomb survivors. Radiat Res 161(3):290–298

Zamarron BF, Chen WJ (2011) Dual roles of immune cells and their factors in cancer development and progression. Int J Biol Sci 7:651–658