INFORMATIVITY OF REGULATORY PROTEINS AT ESTIMATION OF RADIATION-INDUCED CHANGES OF IMMUNE HOMEOSTASIS IN NUCLEAR WORKERS

E.N. Kirillova, M.L. Zakharova, O.S. Pavlova, S.N. Sokolova, T.V.Lukyanova
Southern Urals Biophysics Institute, FMBA 456780, Ozyorsk, Chelyabinsk Region, Ozyorskoe shosse, 19, Russian Federation, tel. (35130) 76370, email: kirillova@subi.su

Risk estimation of severe somatic pathology, including malignant tumors at prolonged radiation exposure, is of great scientific and practical interest [1-4]. Prolonged combined exposure was in conditions of occupational work on Mayak PA radiochemical and plutonium plants. For workers from Mayak main plants (> 500 individuals) exposed to combined radiation (external γ- and internal α- exposure due to incorporated ^{239}Pu) level of ~50 regulatory proteins, participating in immune homeostasis regulation, was studied in blood serum using enzyme multiplied immunoassay: growth factors, multifunctional interleukins, cytokines and their receptors, membrane lymphocyte markers. External γ-doses accumulated during a working career ranged within 0.01-4.9 Gy, and Pu body burden – within 0.03-10.9 kBq. Age of examined individuals was from ~60 to ~80 years, control group included Ozersk population who were not exposed to occupational radiation (examined individuals' gender and age was the same as in Mayak PA workers group).

Growth factors, epidermal (EGF), transforming (TGF-β1), fibroblastic (FGF), hepatic (HGF), platelet-derived (PDGF), as well as multifunctional interleukins (IL-17A, IL-18), cytokines IL-1β, INF-γ and sIL-2R were the most perspective for use as markers of radiation-induced changes of protein status. A Flow Cytometry was used to determine content of effector lymphocyte (B-L, T-L, T-helpers-T-h, T-killers, NK-natural killers) and regulatory cells (TNK with markers T-L and NK, double negative T-L, double positive T-L, with and without membrane markers T-k and T-h).

Increase of level of NK, regulatory TNK lymphocytes, T-k increase and T-h decrease with radiation exposure rise were detected in Mayak workers' blood. This fact gives evidence of tension of T-cell link function and of change in immune homeostasis regulation. Twofold decrease in T-L precursors in comparison with these values in control group was revealed. Relation of regulatory proteins expression from radiation exposure type and level was detected. It indicates immunodeficiency and change in immune homeostasis.

Keywords: immune homeostasis, prolonged exposure, nuclear production

Introduction

Cytokines are molecules which effect on target cell is mediated by high-specific high-affinity membrane receptors. They are responsible for short-distance regulation of intercellular and intersystem interactions. Cytokines influence on survival rate of cells, stimulation and inhibition of their growth, differentiation, functional activation and apoptosis. After interaction between proteins and complementary receptors on cell surface, signal comes to core through elements of intracellular transduction where relevant genes activate. Proteins which are products of activated by cytokines genes, are synthesized by cells and regulate interacting processes. By mechanism, proteins are conditionally divided into growth factors, multifunctional interleukins, pro-inflammatory and anti-inflammatory cytokines and their receptors.

The study objective is to estimate radiation-induced changes in number of regulatory proteins and its role in immune homeostasis disturbance for nuclear workers exposed to prolonged combined radiation in the course of their production activity. For maintenance of immune homeostasis, one of the most important roles is to serum and membrane proteins performing regulatory functions and maintaining homeostasis [5-9].

Level of regulatory proteins of different mechanisms in nuclear workers' serum

Growth factors are polypeptides, like hormones, they have a wide range of biological effect of many cells, stimulate and inhibit mitogenesis, hemataxis and differentiation. As a rule, these proteins are produced by unspecialized cells which are in all tissues, and can be transported to remote target cells with blood flow. Expression level of each growth factor as well as sensibility and type of response are specific for every type of cells [10].

EGF is an epidermal growth factor that controls and stimulates proliferation of epidermal and epithelial cells, including fibroblasts, embryonic cells, participates in wound repair and angiogenesis processes, plays a key role in carcinogenesis, increases Ca release from bone tissue. This protein content in professionals' blood was 1.3 fold lower in comparison with controls (table 1), and concentration decreased with ^{239}Pu body burden increase (figure 1).

TGF-β1 is a transforming growth factor (takes mainly inhibiting effect on immune system, suppresses hemopoiesis, synthesis of inflammatory cytokines, formation of cytotoxic T-L и NK, is secreted by immune system cells). This protein target is various cells because expression of its high-affinity receptor is widely used. TGF-β gene silencing results in development of fatal, generalized inflammatory pathology based on autoimmune process. Level of this growth factor in serum positively correlates with tumor vascularization [6]. Downtrend of TGF-β1 mean group value for workers exposed to combined radiation in a wide dose range (table 1) was registered, and inverse relation between TGF-β1 amount in blood and plutonium body burden was shown (figure 2). Concentration decrease in growth factors with stimulating and suppressing effect on immunocompetent cells indicates rise of immunodeficiency state and reduction of immune control with radiation exposure rise.

Table 1. The level of growth factors in Mayak workers and non-occupationally exposed controls.

Group	Number of persons	Age, year	Dose from external gamma-rays, Gy	Plutonium body burden, kBq	The level of growth factors
EGF, normal level 2.1-76 pg/ml					
Controls	51	56.0±1.2		–	129.2±6.9
Mayak workers	37	66.2±1.2	0.6 ±0.1 0.1÷2.6	1.0±0.3 0.1÷7.0	102.1±6.9*
TGF-β1, normal level 19.0-71.0 ng/ml					
Controls	51	56.0±1.2	-	-	65.2±2.3
Mayak workers	37	66.2±1.2	0.6 ±0.1 0.1÷2.6	1.0±0.3 0.1÷7.0	59.4±2.2
FGF, normal level 0-135 pg/ml					
Controls	22	74.2±1.1	-	-	53.0±1.6
Mayak workers	27	60.8±2.1	0.8±0.1 0.02÷2.6	1.6±0.5 0.03÷11.9	65.1±3.3*
HGF, normal level 475-3083 pg/ml					
Controls	50	56.2±1.2	-	-	959.1±67.4
Mayak workers	37	66.8±1.2	0.6 ±0.10 0.1÷2.6	1.0±0.3 0.2÷7.0	843.8±64.2
IL-18, normal level 78-738pg/ml					
Controls	24	67.9±1.6	-	-	157.9±8.6
Mayak workers	25	72.4±1.0	0.8±0.2 0.1÷4.9	1.6±0.3 0.1÷6.2	128.6±8.7*
IL-17A, pg/ml at normal level cannot be determined					
Controls	17	62.3±2.1	-	-	0.54±0.31
Mayak workers	30	64.3±1.4	0,6±0,1 0.01÷2.0	0,35±0.06 0.01÷1.5	1.39±0.63
Note: *– $p < 0.05$.					

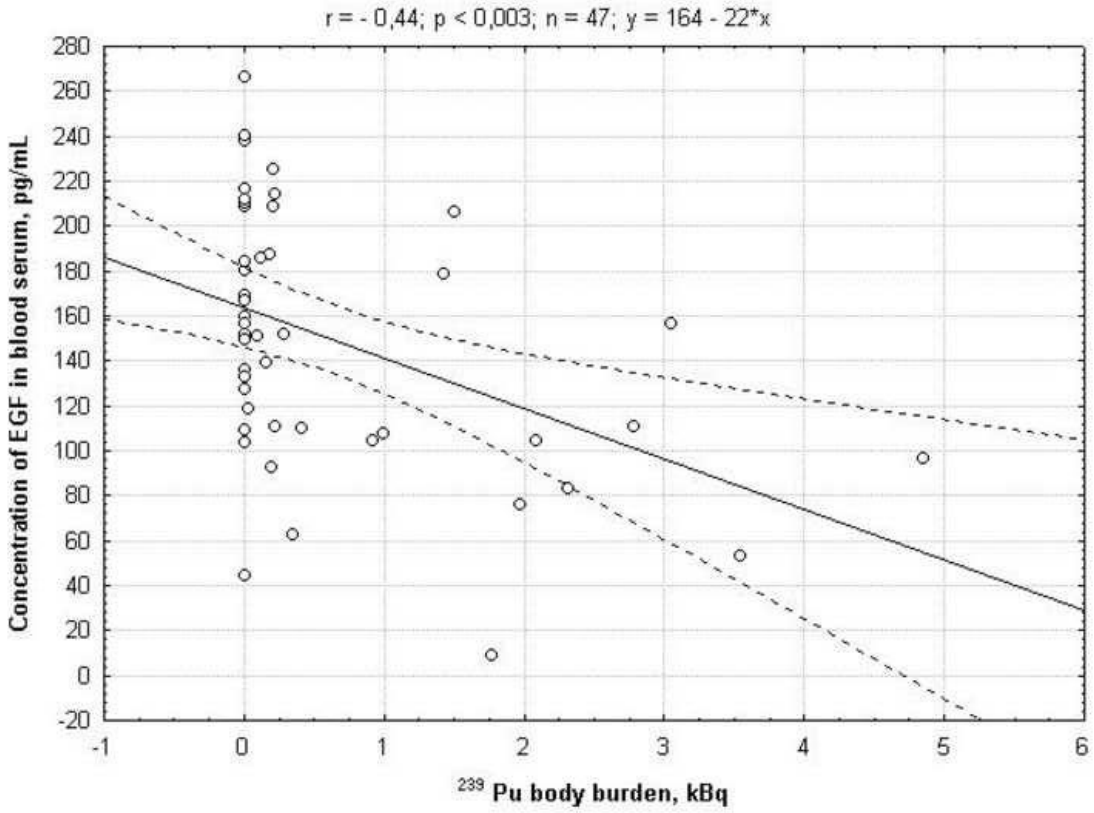

Figure 1. Relation between EGF concentration and examined individual's Pu body burden

FGF biological activity is various. FGF family includes oncoproteins FGF-3, FGF-4, FGF-5, keratinocyte and vascular endothelial growth factors. They are mitogens for different cells of neuroectodermal and mesenchymal nature, potential mitogens and angiogenesis stimulator, maintain and stimulate differentiation of cells of different neuronal types.

In test systems of producer company, FGF concentration values in blood of Mayak PA workers and control individuals did not exceed the bounds of normal, but it should be noted that content of this protein in workers exposed to combined radiation, was more significant (table 1) in comparison with controls.

HGF is a multifunctional cytokine acting like mitogen; it is because of its function in organogenesis and tissue reparation. It is able to stimulate formation of vessels and cell proliferation, therefore, it is supposed to be involved in malignant growth and metastasis in lungs and other organs. In tumor cells, HGF induces bcl-x expression, and, in such a way, inhibits apoptosis. HGF is constantly produced by stromal cells of bone marrow and stimulates hemopoiesis. The level of HGF for individuals in the compared groups was within limits of standard content in blood, but values of average group concentration was lower than in the control, for main plants workers with different doses (table 1). Nevertheless a weak relation (decrease of growth factor) to accumulated external dose was found (figure 3).

Thrombocyte growth factor PDGF is one of potential mitogenetic polypeptides in human blood. α- granules of thrombocytes, macrophages and endothelium cells are PDGF source in blood serum. Structural identity of B-chain and c-sis protooncogene proves that PDGF can have a certain role in virus-induced malignant transformation of infected cells. PDGF takes part in controlling acute inflammation, wound healing and cicatrization processes. PDGF from alveolar macrophages takes part in development of pulmonary fibrosis. It was also established that PDGF is associated with development of atherosclerosis, glomerulonephritis, glomerulonephritis, myelofibrosis and callosity.

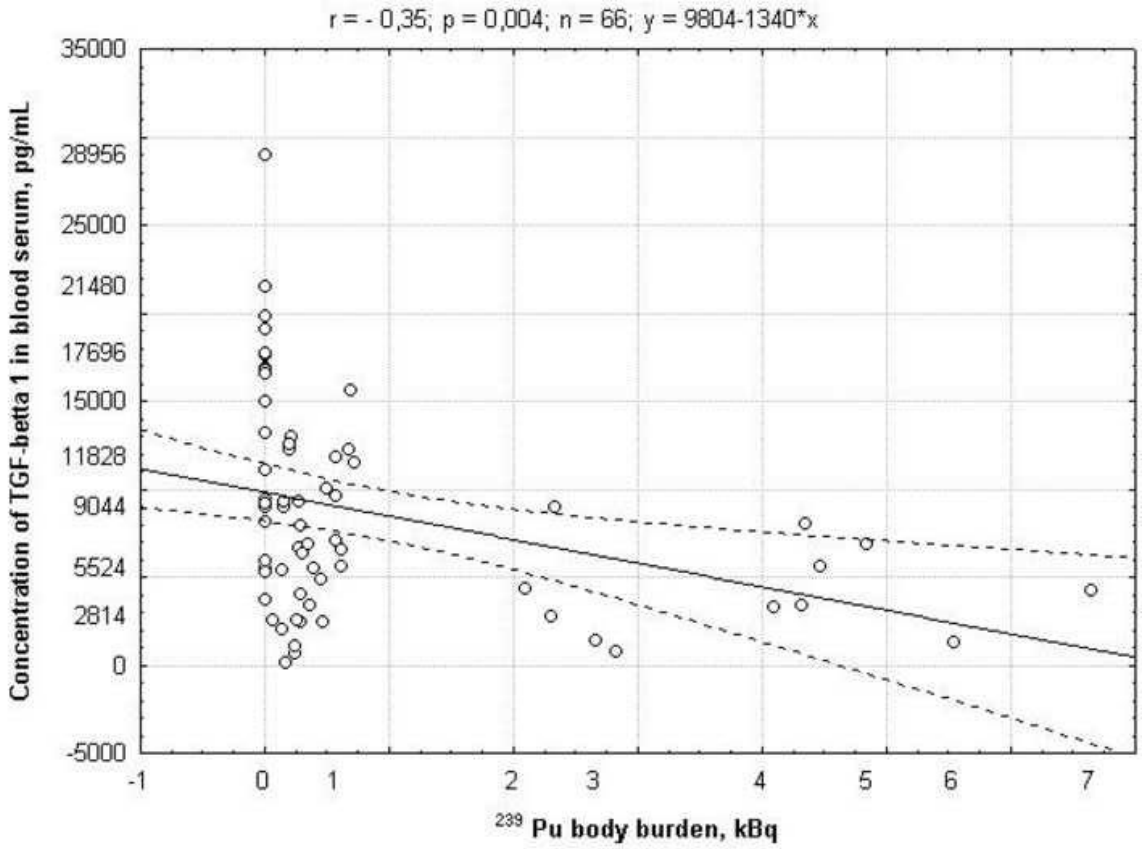

Figure 2. Dependence of TGF-β1 concentration on Pu body burden in examined individuals

It should be noted that for occupational workers average by group content of IL-18 in blood serum decreased significantly by factor of 1.2 ($p<0.02$), compared to the control, whereas decrease of IL-17A level was insignificant due to obvious deviance in certain individuals, but found a direct link to the Pu body burden.

Nuclear workers with combined exposure showed trend to increase of concentration of IL-1β by factor of 2 ($p<0.1$) and direct relationship of increase of the cytokine in blood with external γ-exposure ($r=0.44;p=0.003;n=45;y=40+45*x$) and Pu body burden ($r=36;p=0.01;n=45;y=46.7+40*x$). Increase of IFN-γ content at low dose load was not significant, at higher doses (γ-exposure–1.0±0.9 Gy and α-exposure– 0.9±0.2 kBq) differences in the compared groups were reliable.

Concentration of IL-2 (sIL2R) receptor in blood of exposed individuals was significantly higher than in the control, and in 18% individuals exceeded upper limit of manufacturer's norm by factor of 10. Change of expression of proinflammatory cytokines and their receptors indicate immunodeficiency, related to both adaptive reactions at prolonged exposure, and likely to somatic pathology in elderly individuals.

Numerical estimate of phenotypes of effector lymphocytes

The paper studies contents of effectors and regulators of cell link of innate and adaptive immunity for 233 workers of main plants of radiation-hazardous facility and for 193 Ozersk residents (control).

Workers with exposed exposure had accumulated γ-doses within the range from 0.03 to 7.9 (1.50±0.13) Gy, with ^{239}Pu body burden from 0.03 to 10.9 (1.07±0.19) kBq.

As shown in table 2, level of effectors of adaptive immunity was similar for the main plants workers and control. Content of NK, which are at forefront of antitumor defence, was considerably higher for the nuclear workers, than for unexposed individuals (table 2). Quality assessment of such cells is particularly actual for professional nuclear workers exposed to combined radiation at late period (30-50 years) after beginning of their career at the radiation-hazardous facility. These lymphocytes lyze target cells, including tumor cells without preliminary activation, and also have antibody-dependent cell cytotoxicity. During transformation of cells in the course of development of neoplasms or infection NK

get activated, recognize nonshared antigens on cells membrane and kill them with protein of perforin and granzymes, penetrating to cell target through pores. It should be mentioned that at assessment of number of cells in individuals exposed to combined radiation, in the normal range of accepted values of B-L quantity was in 77.1% of individuals, T-L – in 73.22% of examined individuals, T-h– in 73.5%, T-k – in 58.8%, and NK – in 53.8%, and in 41.7% of individuals NK content was greater than the age rate.
Tumor growth process includes a peculiar selection of tumor cells, i.e. selection of cells containing the least number of specific antigens, as well as with the least antigen properties. Increase of NK amount in elderly people can prove adaptive increase of population of innate immunity effectors for killing mutant cells.

Inductor T-h lymphocytes take part in different immune response: T-h1 are helpers in activation of the effects responsible for function of cell immunity, while T-h2 –for humoral link and differ by set of released cytokines. Correlation analysis revealed relation of T-h quantity with accumulated external dose, i.e. with increase of dose load expression of T-h membrane receptors decreased (figure 4).

As shown in table 2, group average content of T-k, adaptive immunity effectors (also called cytotoxic T-L), in main plant workers did not differ from the one in unexposed individuals of corresponding age. Nevertheless correlation analysis showed trend to increase of this factor in workers with combined exposure (fig.5). Prolonged tension of killer link like adaptive processes in body, and additional body strain (stress, infectious disease),

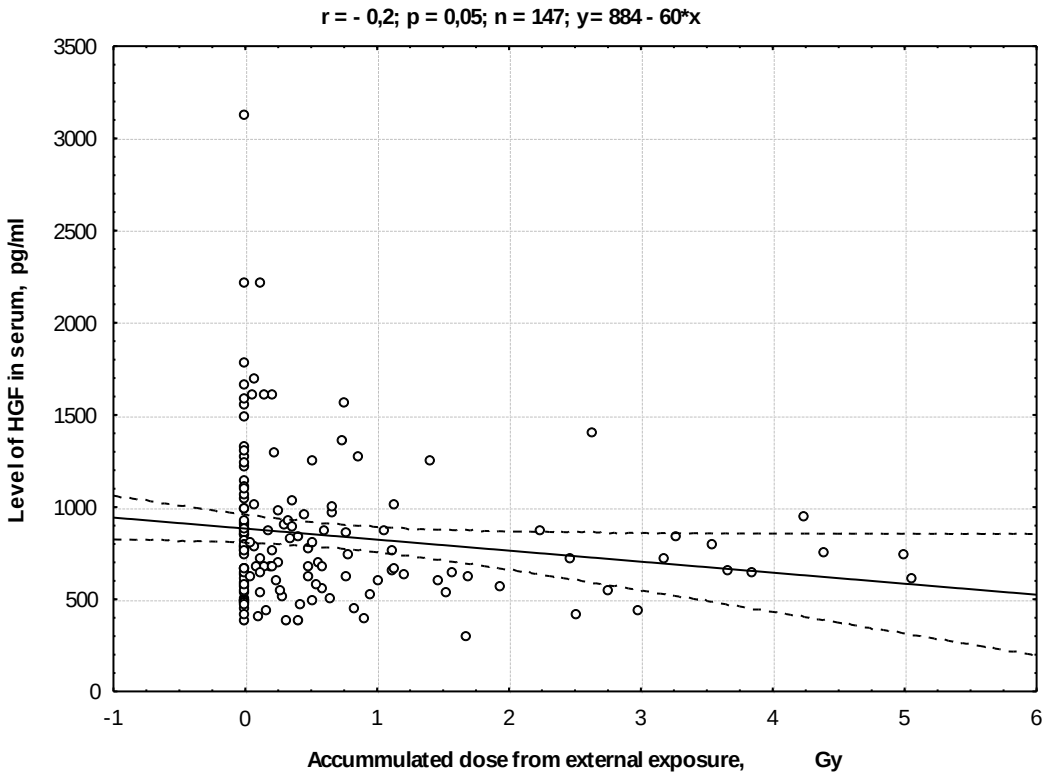

Figure 3. Dependence of HGF concentration on external dose for Mayak workers

Table 2. The level of effector lymphocytes in blood from workers of the main facilities and non-occupationally exposed controls.

Group (number of individuals)	Cells in blood (absolute)				
	B-L (111-376)	T-L (946-2079)	T-h (576-1336)	T-k (372-974)	NK (123-369)
Control	**210.7**±11.8 26÷639	**1619.5**±55.9 360÷2980	**939.6**±33.5 183÷1774	**589.6**±32.7 140÷1630	**313.2**±22.5 63÷1350
Number of individuals	86	86	86	86	86

Main group	**201.0**±10.8 20÷48	**1446.5**±44.2 113÷3616	**827.7**±26,8 302÷1649	**522.5**±22,1 93÷1410	**403.9**±26.2* 37÷1790
Number of individuals in group	84	153	117	153	132
Note: 1.*- differences from control rate are significant, p<0.01; 2. In parentheses – range of test-systems manufacturer's standard values					

ecotoxicology effects etc.), can lead to collapse of adaptation, increase of immunodeficiency, imbalance in content of effectors of different immunity links, decrease of immune control, and result in development of severe somatic pathology, including cancers. Analysis of medical data showed polypathology for elderly examined workers and controls.

Therefore, at prolonged combined occupational exposure number of effectors of innate immunity NK increases, and there is dependence of content of effectors of cell link of adaptive immunity on type and level of radiation effect (T-k and T-h).

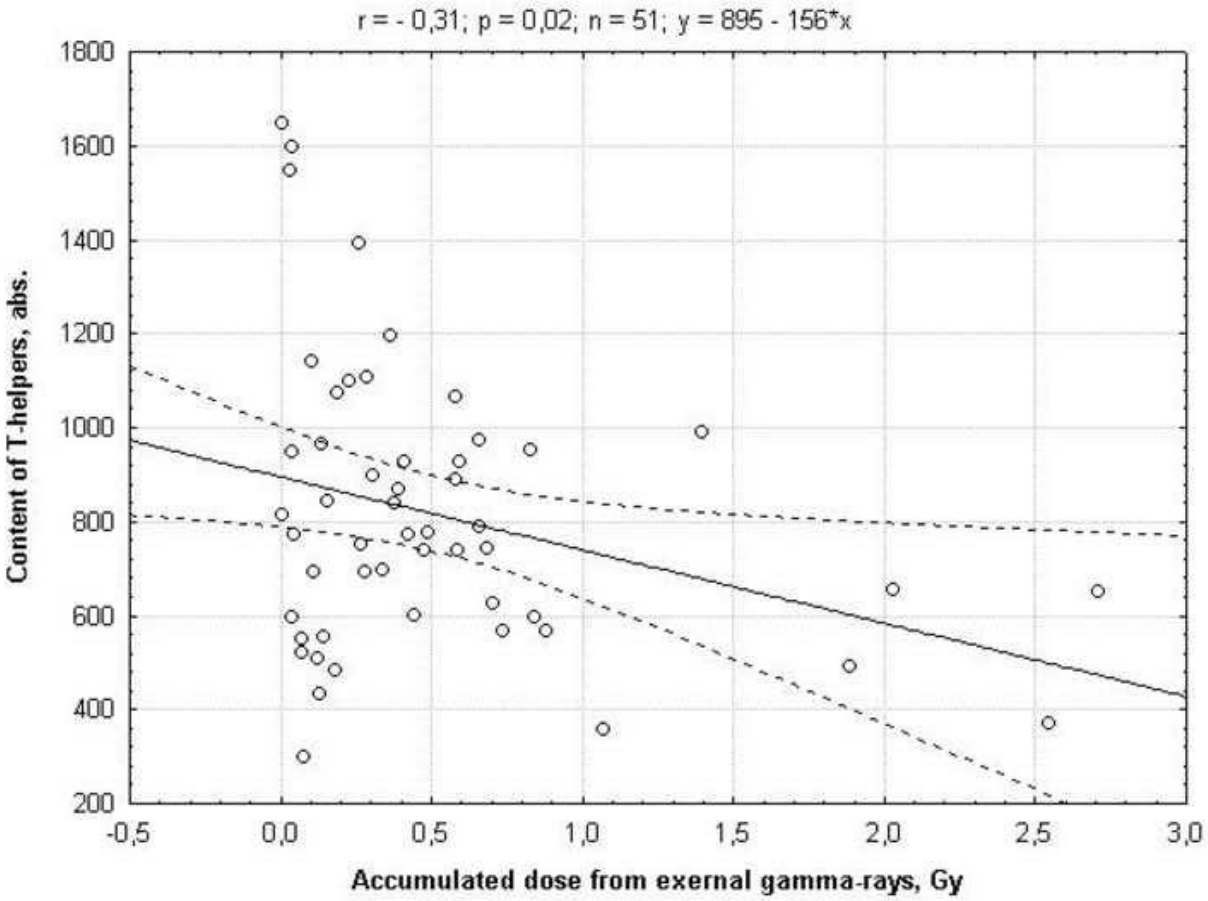

Figure 4. Dependence of T-h number on the accumulated external dose in the studied individuals.

Content of regulatory lymphocytes in Mayak PA main plant workers and their role in maintaining immune homeostasis at long-term exposure.

It should be noted that at a young age a large part of T-lymphocytes is replenished mainly due to the inflow of naïve T-cells from the thymus and in a less degree of memory T-L. In elderly individuals homeostatic proliferation is effected mainly due to memory cells [9].

The number of young forms of T-L (early stages) able to regulate the proliferation and differentiation of cells – double negative lymphocytes (double negative T-L) in workers exposed to combined exposure decreased almost 2.5 times (Table 2). These cells are devoid of membrane markers T-h and T-k. Almost the same decrease at combined exposure was revealed at the analysis of more mature cells content in T-L population – double positive T-lymphocytes (double positive T-L), which contrary to double negative T-L acquired markers T-h and T-k (Table. 2). Afterwards these cells divide into 2 subpopulations and become single positive. In this period they lose the marker of progenitor of cells CD38 and receptor of transferrin. The decrease in number of T-L regulatory progenitors in individuals exposed to prolonged occupational radiation exposure is indicative of a deeper reduction of committed T-cells department compared to controls.

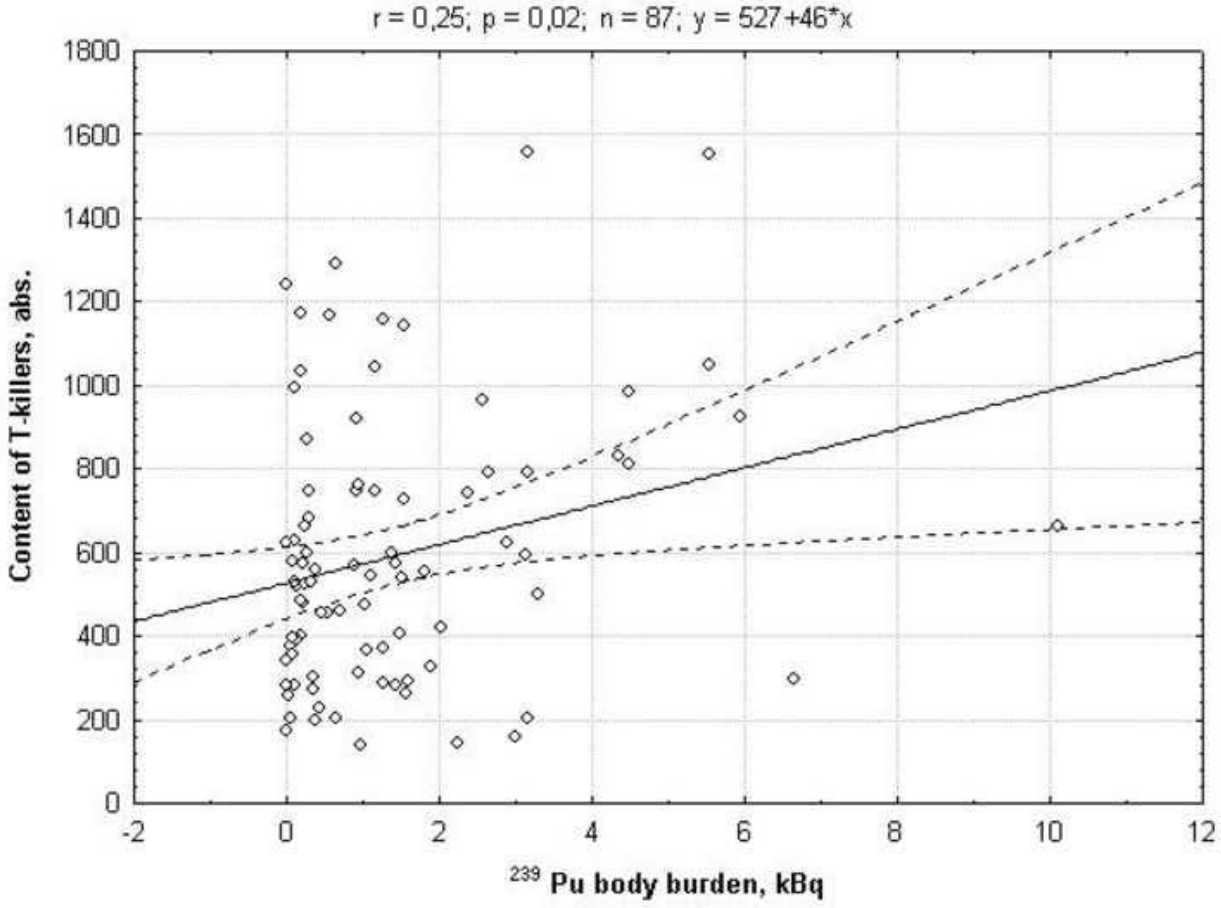

Figure 5. Dependence of T-k number on Pu body burden in the studied individuals.

Table 3. The level of regulator lymphocytes in blood from workers of the main facilities and non-occupationally exposed controls.

Groups	Cells in blood (absolute)		
	NKT (7-165)	T-L Double+	T_L Double-
Control	**75.7**±6.8 12÷307	**99.9**±8.4 28÷266	**155.5**±13.6 26÷392
Number of individuals in a group	58	41	41
Mayak workers	**150.2**±17.0* 5÷904	**51.7**±8.5* 5÷285	**63.5**±8.8* 9÷236
Number of individuals in a group	68	46	39

Note:
*- differences from control rate are significant, $p<0.05$;

Correlation analysis of the indices obtained at the follow-up of workers exposed to combined radiation exposure showed the inversed dependence of the number of young T-lymphocytes of different degree of maturity on radionuclide body burden. The decrease of the number of double positive T-L with the increase of accumulated external dose (r= -0.28, p=0.008; n=87, y=83-33*x) and the tendency to decrease with the increase of Pu body burden (r= -0.20, p=0.08;n=87, y=80-7*x) was found. The significant decrease of the number of double negative T-L with the increase of accumulated dose both from external radiation exposure and Pu body burden (r= -0.3.p=0.007; n=80y=123.4-15.3*x; and r= -0.38.p=0.0006; n=80y=127.5-56*x) was shown.

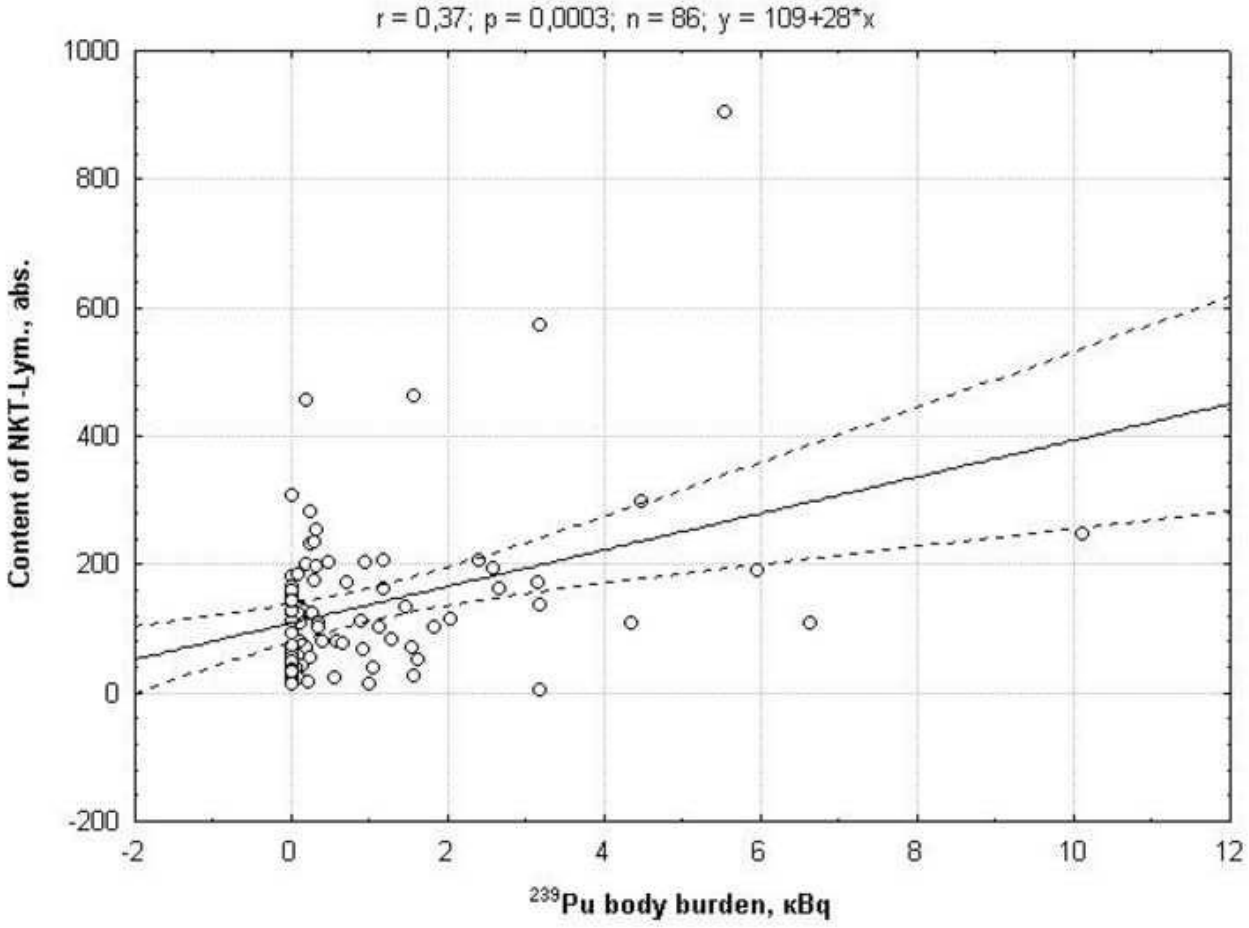

Figure 6. Dependence of TNK number on Pu body burden in the studied individuals.

Recently, the properties of TNK-lymphocytes have been studied. They combine T-L and NK markers on their membrane, which have cytotoxic activity, initiate T-cell response and act as means of communication between the systems of inborn and acquired immunity. TNK-cells are considered to be able to regulate immune response (in particular, functions of dendritic cells) by produced cytokines, for example IL-10 [60]. The study of the content of these cells in blood showed twofold increase of their mean group index at combined exposure (Table 2) compared to controls.

The search of biomarkers of prolonged combined radiation exposure late effects in professionals of nuclear enterprises is a particularly important and complex task. The reveled indicators of radiation effects will be recommended to practical public health service for expert evaluation of harm to the health as a result of occupational exposure. Immune system is of great homeostatic importance to the organism vital functions. The knowledge of the mechanisms of damaging effect of ionizing radiation on immunocompetent cells, cellular cooperation, protein regulation of immune response enables to evaluate the nature of early and late post-radiation immunodeficiency. The forming of risk groups, development of

personified protein map and immune status control will allow improvement of early detection, prophylaxis and expert care of occupational exposure effects.

Conclusion

Changes in immune and protein status were revealed for workers from the main nuclear plants in prolonged period of occupational exposure. Compared to values for non-exposed during their professional activity individuals, mean group number of effector lymphocytes of different phenotypes in professionals' peripheral blood was not significantly different. Natural killer effectors of genetic immunity participating in antitumor and anti-infectious protection were an exception. Content of them was significantly greater for nuclear workers compared to controls. Deviations from normal expression of membrane receptors of adaptive immune effectors were not registered for 70-80% professionals, however, during the correlation analysis, relation between T-helpers with external accumulated dose (decrease in cells number with increase in radiation exposure level) and T-killers (increase in lymphocytes number with increase in ^{239}Pu body burden), was revealed. At the same time, change in regulatory lymphocytes content in blood was registered for workers compared to controls: increase in TNK amount with T-L and NK markers as well as decrease in T-L young regulatory precursors of early (double negative T-L) and late (double positive T-L) differentiation stage. These observed changes of immune status indicate not only adaptive change in immune regulation but immunodeficiency, increasing dose load.
Regulatory proteins are integrated part of extracellular signal net that controls all functions of genetic and adaptive immunity, they effect on formation of cell phenotype. In serum obtained from nuclear workers, a study of level of ~ 50 regulatory proteins of different mechanism was carried out. The most perspective for use as markers of radiation-induced changes in protein status were epidermal, transforming, fibroblastic, hepatic, platelet-derived growth factors as well as multifunctional interleukins (IL-17A, IL-18), proinflammatory cytokines IL-1β and INF-γ, and soluble receptor IL-2. Imbalance of growth factors related to radiation type and dose load that are responsible for proliferation, differentiation and apoptosis of cells as well as activation of proinflammatory cytokines indicate radiation-induced change in protein status and decrease in immune control. Therefore, decrease in antitumor body resistance in different extreme and stress situations can result in activation of malignant transformation of cells, accelerated growth of malignant tumors, development of cardiovascular diseases and other serious somatic pathology. So long as medical data were obtained by the time of blood collection for all examined individuals, from our point of view, the study of relation between change in studied values and disease incidence is extremely important.

This work has been performed with the support of:
- Federal Medical Biological Agency (FMBA) of the Russian Federation
- U.S. Department of Energy, Office of International Health Studies (HS-14)

References

1. Tokarskaya Z.B., Khokhryakov V.F., Khokhryakov V.V., Kirillova E.N., Vasilenko E.K. Journal of Radiological Protection. 2010. 55.13-32.
2. Preston D.L., Krestinina L.Y., Sokolnikov M.A., Ron E., Davis F.G., Ostroumova E.V., Gilbert E.S. Radiat. Res. 2010.174. 816-824.
3. Sokolnikov M.E., Gilbert E.S., Preston D.L., Ron, E., Shilnikova N.S., Khokhryakov V.V., Vasilenko E.K., Koshurnikova N.A. International Journal of Cancer 2008. 123. 905–911.
4. Azizova T.V, Muirhead C.R, Druzhinina M.V, Grigoryeva E.S, Vlasenko E.V, Sumina M.V, O'Hagan J.A, Zhang W, Haylock R.G.E, Hunter N. Medical Radiology and Radiation Safety. 2010. 55. 5. 14-23.
5. Kirillova E.N., Zakharova M.L., Drugova E.D., Pavlova O.S. Russian Allergology Journal. 2010. 5. 137-138.
6. Souchelnytskyi S. Experimental Oncology 2002. 24. 3–12.
7. Sviridova V.S, Kologrivova E.N, Pronina N.A. et al. Bulletin Siberian Med 2007.1. 83-88.
8. Khaitov R.M. Allergy, Asthma Clinic Immunol. 1999. 1. 6-20.
9. Kleemann R., Zadelaar S. and Kooistra T. Cardiovascular Research. 2008. 79.360-376.
10. Male D., Brostoff J.Rot D.B., Roitt I. Immunology. 2007. Moscow. (Logosphera). 127-235.