

IMMUNOBIOLOGY AND HEMATOLOGICAL ASPECTS OF HEALTH - 10 YEARS AFTER CHERNOBYL DISASTER

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INTRODUCTION

The Chernobyl Accident led to special situation when some people taking part in elimination of its onsequences of Chernobyl accident were irradiated in high doses. 145 men had suffered from acute radiation syndrome of different degree, and 105 of them lived in Ukraine. Millions of Ukrainians were also suffered from other accident consequences as a clean-up workers, inhabitants of highly contaminated territories etc. Immune and hematologic disturbances played the important role in early reactions to irradiation and other factors [1 - 3]. They are very important still in predicting late health effects 10 years after the Chernobyl accident.

PATIENTS AND METHODS

Forty two thousand of patients who belonged various categories of irradiated population were studied during 10 years after the accident. Chernobyl acute radiation syndrome patients, clean-up workers who were exposed to the doses under 1 Gy limit in 1986 and subsequent years, children and adults evacuated Prypjat, 30-km zone and contaminated territories population were the groups of first-line interest.

Immunologic studies included flow cytometric determination of immunologic phenotype with Leu IMK, IMK Plus, Simulset monoclonal antibodies (MoAbs) kits (Becton Dickinson, CA, USA) in two-color assay, DNA cytometry and cell cycle analysis, activation studies (tetracycline incorporation, IL-2R, TrR expression, BRDU-, 3HTdR-uptake) in perturbed and non-perturbed cell populations, also serum Ig content, anti-RBC, anti-WBC and anti-platelet agglutinins and lysins. Radiation induced variant cells content was investigated in RBC GPA-expression assay from 1990, TCR-mutations assay from 1992. Search of genetic basis of radiosensitivity was performed in HLA-A, B, C, Dr distribution (Dr J.Minchenko). Patients evaluation was supported by IPHECA WHO project from 1993. HbsAg and HbC prevalence studies in irradiated population were conducted with the help of Dept. of Epidemiology, Hiroshima Univ.(prof.H.Yoshizawa). 'Comet' DNA assay was introduced in 1995. Hematological studies included peripheral blood, bone marrow cells morphologic and cytochemical examination by means of light and electron microscopy, studies of microenvironment, progenitor cells and fibroblast cultivation, electron-paramagnetic resonance, infra-red spectroscopy etc. Dose dependent estimation was available with the help of dosimetric departments of Chernobyl NPP, Instituts of Experimental Radiology and Epidemiology of RCRM. Cytogenetic studies of acute radiation syndrome patients in 1986 were performed in Medical Radiology Centre in Obninsk. Clinical investigation and laboratory data analysis were performed with the help of standardized criteria.

RESULTS AND DISCUSSION

A three stage process of the immune system recovery was seen in persons who suffered from external acute irradiation. Dynamic investigations of immune state revealed a post radiation deficiency. The cellular or/and humoral immunity disturbances included surface phenotype changes, especially in mitogen-associated populations of CD3+, CD4+ and NK- cells (CD57+, CD11+16+). It was worth to mention the mosaic of injury and reparation of surface membranes of immunocompetent cells. B-cells exhibited the short-time decrease of C3+ and pan-B-antigen bearing cells. Membrane changes also evoked the difficulties in locus A,B,C HLA-typing. A study of T-cell receptor stable alterations was performed among the population of contaminated territories and personnel of the Chernobyl station in 1990 -1992. A two-fold (0.4 - 0.6 per cent of the PMNC) increase of aberrant cells with decreased TCR expression was found in investigated contingents. It was accompanied by the 2 -3 fold increase of GPA aberrant RBC. The duration of first stage was from 6 to 24 mnths, the absorbed dose and the duration of irradiation, the presence of somatic and psychosomatic

pathology, age at the moment of irradiation have influenced greatly. At the second stage was the restitution of the radiation injury the increase of subpopulation of CD3+DR+ lymphocytes was seen, accompanied by stable tendencies of finding in the peripheral blood of lymphocytes with phenotype CD4+8+ and CD1+, which normally were characteristic only for intrathymic stages of differentiation. The increase of B-lymphocyte count, which had pan-B-markers as CD21, C3, late differentiation markers such as surface IgG and early antigens as CD10. HLA-typing revealed the prevalence of HLA A1, 28; B 5,38; B 6, 17; B17, 18; B8,22; B 8, 27; B 27, 35; A1, B16; A1, B27; A2, B38; A10, B38; A 28, B 8 in patients who had suffered from acute radiation sickness. These data are very important for the estimation of the radiosensitivity in individuals exposed to the lesser doses and for professional selection. HLA-A1,9; B8,12; B12,13; B15,35 antigens were associated with thyroid pathology both in contaminated and clear regions of Ukraine but no differences depended on radiation. Five-six years after the irradiation a heterogeneity of types of immunologic response of acute radiation sickness reconvalescents was detected. A group of patients demonstrated normalisation of CD3+, CD4+, CD11+ cell count and serum IgG and IgA content, while the others revealed immunologic deficiency of the mixed type. Subset cell cycle analysis with PJ showed the decreased response to Con A in 18 hour cultures as well as 3H-Tdr uptake in 72 hours cultures. Dose-dependent changes of enkephaline receptor on PMNC and sensibilisation to brain antigens accompanied by changes of TrR, RIL-2, CD10, CD23 activation antigens expression in healthy irradiated persons as well. Late radiation subset and functional effects could be explained by neuro-humoral regulatory changes. Late effects included compensation of radiation injury in 32% of patients. High CD3+19-, CD4+8-, CD16+56+ cells counts were accompanied by low proportion of CD8+4- cells. Non-specific activation of T-link was revealed with high contents of CD3+ and CD4+ cells, exhibiting HLADR antigens and high IL-2 and Transferrine receptors expression. Non-specific activation, especially of B-cells could be the basis of immunopathologic processes in the future. Compensatory changes were associated with the low mutation rates in T-cell receptor complex.

Dysregulatory changes were detected in 37% of patients. Individualisation of immune disorders, changed response to neuro-humoral stimuli and wavy variations of immune parameters were accompanied by the low variant CD3-4+ cells incidence - less than 5 cells/10000. Depression of cellular immunity developed as the decrease of compensation abilities especially in T-cells.

During the investigations of the mechanisms of radiation-induced pathology of haemopoietic system and role of haemopoietic cells and its microenvironment in creation of the distant irradiation consequences among reconvalescents of ARS different qualitative modifications of peripheral blood and bone marrow cells were found. Hypersegmentation, fragmentation of nucleus, the toxical granulation, fringe and basophilia of the lymphocyte's cytoplasm, the vacuolisation of cytoplasm and nucleus were found. The increase of bone marrow cellularity about $10,0 - 13,0 \times 10^9 / l$ in 8 patients of examined group was determined. For the other 14 people the number of myelocaryocytes was normal and the hypercellularity was found in 7 patients. The number of megacaryocytes fluctuated, as a rule, on the level of physiological standart, but the increase of the number of megacaryocytes with less size of nuclear and its shape modification was found. The number of cells with active production of platelets was decreased.

The correlation between the hematopoietic lineages was normal in the most cases in spite of the number of myelocaryocytes have decreased. Four of the patients had the narrowing of erythroid lineage from 5,5 to 10,5 %. For only one patient with myelodysplastic syndrom the irritation of erythroid lineage about 42% was observed. Three of the patients had lymphocytosis in the bone marrow about 33-34%, two of them had eosinophilia about 5%.

The membrane permeability of blood erythrocytes was increased in the investigating group according to the data of osmotic and mechanical resistance of erythrocytes. The results of studying of surface membran adsorption ability and its volume showed the considerable modifications of these indices. The thorning and spherical shapes of erythrocytes were found in the peripheral blood of 40 % observed patients. Bone marrow paramagnetic centres studies showed that intensity of index of bone marrow paramagnetic centres differed from one in blood in persons with steadfast deviations of peripheral blood. These results testify to significant modifications in function of important metalloproteides - transferrine and ceruloplasmine, which carry out iron transport in bone marrow, in each specific case.

The restitution of the homeostatic characteristics of hemopoetic and immune systems occurred at the deviated level of regulation especially in patients with high absorbed doses; individual radiosensitivity could be revealed in the low dose limit as well as in radiation pathology. Comprehensive investigation of various exposed groups led to the opinion that the control of cell differentiation could be the key point in late effects of irradiation. Still the quantity of questions is overwhelming.

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