Aberrant Lymphocytes and Sensitivity to Apoptosis at Low Doses of Radiation

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The Chernobyl accident is unique by its scale and by the characteristics of the radiation spectrum. Careful assessment of the medical consequences of Chernobyl accident requires taking into account its various unique features including the wide spectrum of released radionuclides and the election of hot particles of nuclear fuel as well as action of some other factors.

The main threat by the accident especially to children was caused by the iodine isotopes because of their tropism to the thyroid gland. The absence of iodine prophylaxis, the non-compliance with the norms of behavior in the case of radiation danger and the delay of evacuation have entailed the overdose irradiation both of thyroid and the whole body [6,16]. Later after relocation to the areas of relatively low radiactive contamination the children still have been constantly exposed to the low doses of the internal and external radiation. Unfortunately the individual dose equivalent obtained by each child practically cannot be reconstructed precisely.

Material and methods

Using the hematological automatic system MINOS STX (France) 7250 children, who were evacuated from Prypyat city soon after the Chernobyl Power Station accident were studied during the years 1994-1995. All children were evacuated in May 1986 to Kyiv and live there since that time. The average dose equivalent for this group of children can be evaluated on the basis of recently published data [1, 11, 16].

The cytomorphologic peculiarities of leukocytes in the smears stained according to May-Grunwald-Giemsa were studied in children with quantitative deviation of blood indices from normal in corresponding age groups. When abnormal or atypical cells were revealed the cytochemical and immunocytochemical spot checks were carried out.

Blood samples. Venous blood smears for morphological and cytochemical study were prepared by routine methods. Furthermore a mononuclear cell fraction was obtained from some samples by centrifugation in Ficoll-Verografin density gradient (d = 1.076-1.078). These cells were centrifuged onto the clean glass slides. Smears were frozen at -200 C before the immunocytochemical staining.

Cytochemical staining. The intracellular localization and activity of acid phosphatase by the method of azocoupling according to Goldberg and Barka, of acid non-specific esterase according to Muller et al and the content of PAS-positive substances were studied [14]. The blood smears were fixed for 3 min in vapours of 10% neutral formaline.

Monoclonal antibodies. The monoclonal antibodies (MoAbs) LT1 (CD5), LT3 (CD3), LT7 (CD7), LT4 (CD4), LT8 (CD8), LT45RA (CD45RA), LNK-16 (CD16) [5], DAKO-CD19 (CD19), UCHL-1 (CD45RO), DAKO-IL-2 (CD25), DAKO- Transferrin (CD71), DAKO-CD10 (CD10) (Dakopatts, Denmark), IPO-4 (CD95) [17], ICO-1 (HLA-DR), ICO-12 (CD22) [2] were used.

Immunocytochemical staining. Cytocentrifuged cells were stained by a three-stage immunoperoxidase (PAP), immunoalkaline

(APAAP) procedure or streptavidin-biotine-peroxidase method [14]. Before staining the cells were fixed for 3 min and rinsed in buffer.

Apoptosis study. In 1995, the sensitivity to apoptosis at low doses of radiation was studied in a group of 21 adult persons, who had been at the time of the accident at the Chernobyl nuclear power station and during the next two days in Prypyat (4 km near the epicenter of the Chernobyl catastrophe), and now they are constantly living in Kyiv. 11 men (22-45 years old, average 38) and 10 women (32-48 years old, average 40) were the members of this group. A Control group was constituted from 15 healthy persons born and grown up in Kyiv (8 men, 29-49 years old, average 37 and 7 women, 39-42 years old, average 39). Somatic diseases in these groups were not revealed.

Peripheral blood mononuclears were isolated by centrifugation in the density gradient of Ficoll-Verographin, washed with Hanks medium and incubated for 24 hours at 37° in 5% $C0_2$ in complete medium RPMI-1788 with 10% of calf embryonic serum ("Sigma", USA), 100 ED penicillin and 100 ED streptomycin.

Induction of apoptosis program in lymphoid and hematopoietic cells was realized by adding anti-Fas MoAbs IPO-4 (1 mg/ml) or dexamethasone (0,1 and 0,5 mg/ml). After incubation a number of perish cells was calculated using trypan blue solution, and a number of apoptotic cells with condensation of chromatin was estimated by dyeing for 30 min at 37° with vital fluorescent dye Hoechst 33342. In the examination the apoptotic cells showed bright fluorescence and irregular structure of nuclei [18].

Results and discussion

In 1275 of 7250 children observed the quantitative deviations of studied blood indices from normal ones in corresponding age groups were determined. They were manifested as moderate leukopenia, more seldom as leukocytosis, thrombocytopenia, normochromic anemia. Eozinophilia, relative and absolute lymphocytosis, and an increase of monocyte content were often noted. Also remarkable were such features as an increase in the number of large hypersegmented granulocytes, fragmentation of neutrophil nuclei, appearance of giant platelets, change of small to large lymphocyte ratio, and an appearance of immunoblasts and mononuclears. Also there were detected lymphocytes with sings of plasmatization and with vacuolized cytoplasm. In some children a portion of lymphocytes showed the hairy processes.

In 631 of 1275 children (49,5%) abnormal lymphocytes with nuclear alterations were found in blood smears. Light microscopic studies showed multiple nuclear indentations and radial segmentation. These lymphocytes accounted for 3,3-39,5% of all lymphocytes. In some cases multilobulated nuclei in lymphocytes were similar to cell nuclei observed in a variety of different T-cell lymphoid neoplasms. In contrast, nuclei of B-cell lymphomas showed single clefts. Lymphocytes with such nuclear alterations were absent in the peripheral blood of 380 children of control group residents of Kyiv before April 1986. We have not found them earlier in the smears of patients with different diseases.

Lymphocytes with nuclear indentations and radial segmentation (2-8%) have been observed also in 25 of 48 children born before 26 January 1987 who were exposed to radiation in uteri, and in 34 of 45 children born 9 month or more after the father's and mother's (or both) exposure to ionizing radiation due to the Chernobyl accident.

On the surface membrane of the lymphocytes with nuclear alterations the expression of pan-T-cell CD7, CD3 and CD5 antigens and CD45RO antigens was de-

termined. Aberrant lymphocytes were detected among the phenotypic subpopulation of T-helpers/inductors (CD4+ cells) (10-12%) as well as T-supressors (CD8+ cells) (15-18%). Approximately in half of the lymphocytes with abnormal nuclei the CD16 antigen (receptor of Fc-fragment of IgG) expressed on the natural killer cells (NK-cells), granulocytes and macrophages was detected. At the same time the CD16+ cells represent 25-27% of usual small and medium size lymphocytes of peripheral blood. 15-25% of the aberrant lymphocytes expressed T-cell receptors with γ and σ chains, of preferentially nondisulfide-linked form. Aberrant lymphocytes do not react with anti B-cell MoAbs CD22, CD19 and HLA-DR, and do not contain the CD10 antigen determined on the pre-B-cells and on the B-cells of germinal centers of the lymphoid follicles of lymph nodes and of the spleen.

Based on the character of deposit and the activities of acid phosphatase and acid nonspecific esterase the aberrant lymphocytes differ from ordinary T-cells.

Morphological changes in the nuclei of cells (fragmentation, multilobation etc.) are sensitive indicators of the effect of low dose radiation [4, 7, 8]. The detection of lymphocytes with abnormal nuclei in the blood of children 5-10 years after their exposure due to the Chernobyl accident (with the following low dose exposure) can be explained by the fact that in consequence of the antigen stimulation the T-lymphocyte resistance to the interphase death was sharply increased. This superresistant population averaged 3-5% of blood lymphocytes. The presence of chromosome aberrations appearing on exposure to low doses of radiation and concominant disturbances of nuclear structure seemed not to hinder the long-term existence of such lymphocytes in lymphoid organs and peripheral blood. It is worth to note that in some cases the number of detected cells with abnormal nuclei corresponded to the reported frequency of chromosome aberrations in the lymphocytes of children living in the regions of radioactive fall-out after the Chernobyl accident [13]. The appearance of aberrant lymphocyte subpopulations may be connected not with direct radiation damage of DNA but with the activation of lymphotropic viruses having been postulated to play a role in the pathogenesis of autoimmune diseases [3]. It is not excluded that the appearance of lymphocytes with "pathologic" nuclei may be the consequence of a transmission of non-lethal damages in the genome of progenitor cells to the next generations in the process of lymphoid cell differentiation. It is quite possible that the structural changes in T-cell nuclei being revealed several years after the irradiation could result from radiation damage of auxiliary cells of the immune system, the stromal cells of thymus in particular upon the initial exposure with following disturbances of T-lymphocyte maturation. On the basis of immunophenotyping with MoAbs the abnormal lymphocytes were found to belong to the population of T-lymphocytes or NK-cells, the main effector cells of anticancer immunity. Therefore, a possible prognostic significance of these data can be assumed bearing in mind the increased incidence of thyroid cancer and childhood acute leukemias [12, 15] as the most probable outcome of the exposure due to the accident at the Chernobyl nuclear power plant.

Blood mononuclears of healthy persons, who composed a control group in our investigation, were resistant to apoptosis induced by dexamethasone and MoAbs IPO-4 (table1). On the contrary, blood mononuclears of persons, evacuated from Prypyat soon after the Chernobyl nuclear power accident, were sensitive to the action of inductors of apoptosis. It was manifested by the increase of a number of living cells with specific morphological signs, chroma-

tin condensation and bright fluorescence after Hoechst 33342 dyeing.

For elucidation of the feasible reasons for the sensitivity of blood mononuclears in Chernobyl victims we investigated lymphocyte subpopulations in them. It is known that separate lymphoid fractions - for example, CD45RO+CD4+ peripheral blood cells from patients with infectious mononucleosis [19], and HIV-infected cells [9], are subjected to apoptosis to a greater degree than others. We did not find any significant distinctions in the frequency of expression of studied antigens on the membrane of lymphocytes from healthy persons and Chernobyl victims (table 2). In both groups a correlative dependence between the number of CD95-positive cells and the sensitivity of blood mononuclears to anti-Fas MoAbs was absent ($r = 0.351$ in the group of Chernobyl victims and $r = 0.385$ in the group of healthy persons, $p>0.05$). Lymphoid cells from all examined persons did not express activating antigens CD25 and CD71 and antigen CD10 (marker of separate B cell subpopulation of bone marrow and lymph nodes).

The sensitivity of blood mononuclears of Chernobyl victims to apoptosis did not associate with the changes of lymphoid subpopulations, in particular an increase of the number of CD45RO+ and CD95+ cells, and the appearance of activated lymphocytes. It permits us to suggest that the fraction of cells in the peripheral blood of liquidators bearing corresponding receptors to inductors of apoptosis is not quantitatively increased, but is qualitatively changed - the intracellular mechanism of signal transmission leading to programmed death is not blocked in lymphocytes of Chernobyl victims in contrary to lymphocytes of healthy persons, who also have necessary receptors. It is interesting that in the bone marrow of some liquidators we found a significant number of alkaline phophatase-positive dendritic cells. Until 1986 such cells were detected only in bone marrow smears of patients with cancer metastases affection. AlP+ dendritic cells contained vimentin filaments, HLA-DR, HLA-ABC and CD10 antigens. Other surface antigens (CD45, CD34, CD33, CD13, CD71, CD38, CD54, CD14, CD15, CD64, CD68, antigens of T- and B-cells) were absent. The relation of AlP+ stromal cells with sensitivity of bone marrow mononuclears to apoptosis (analogous to the role of follicular dendritic cells of lymph node) will be the subject of our future study.

References

1. Anspaugh LP, Catlin RJ, Goldman M.: The global impact of the Chernobyl reactor accident. Science 1988; 242: 1513-1519.

2. Baryshnikov AYu, Kadagidze ZG, Machonova LV, Tupitsin NN.: Immunological phenotype of leukemia cell. Moscow, Medizina, 1989 (in Russian) - 164 p.

3. Bessis M.: Living blood cells and their ultrastructure. Berlin, Springer, 1973. - 767 p.

4. Dobson RL, Chupp MM.: Effect of small measured doses of radiation on lymphocyte morphology in man. Proc. Soc. Exp. Biol. Med. 1957; 95: 360-361.

5. Filatov AV, Scherbukhin VV, Bachurin PS.: Two-colour cytometric study of lymphocyte subpopulations in patients with primary immunodeficiences. Biomed. Sci 1992; 2: 88-96.

6. Health effects of exposure to low level of ionizing radiation. BEIR V. Washington, Natl. Acad. Press, 1990. - 421 p.

7. Ingram M, Adams M, Coonan L.: The occurence of lymphocytes with bilobed nuclei in cyclotron personnel. Science 1952; 116: 706-708.

8. Jacobson LO.: The effect of ionizing radiation on blood and hematopoietic organs. Radiation Biology. High Energy Radiation. New York-Toronto-London, McGrawy Hill Book Company, 1954: 251-317.

9. Kobayaski N, Hamamoto Y, Yamamoto N.: Anti-Fas monoclonal antibody is cytocidal to human immunodefixiency virus-infected cells without augmenting viral replication. Proc. Natl. Acad. Sci 1990: 87: 9620-9624.

10. Miyawaki T, Uchara T, Nibu R.: Differential expression of apoptosis related Fas antigen on lymphocyte subpopulation in human peripheral blood. J. Immunol. 1992; 149: 3753-3758.
11. Norman C, Dickson D.: The aftermath of Chernobyl. Science 1986; 233: 1141-1143.
12. Parkin DM, Clayton D, Black RJ.: Childhood leukemia in Europe after Chernobyl: 5 year follow up. Br.J.Cancer 1996; 73: 1006-1012.
13. Pilinskaya MA, Shemetun AM, Bondar Ayu, Dobsky SS.: Cytogenetic effect in somatic cells of people, irradiated after the Chernobyl accident. News of AMS USSR 1991; 8: 40-43.
14. Pinchouk VG, Gluzman DF, Nadgornaya VA.: Immunocytochemistry and monoclonal antibodies in oncohematology. Kyiv, Naukova dumka, 1990 (in Russian) - 201 p.
15. Schmitz-Feuerhake I.: Occupational risk from radiation, in Eylenbosch, W.J., Van Larebeke, N., Deporter, A.M. (Eds.), Primary Prevention of Cancer 1988: Raven Press, New York, p. 129-139.
16. Serkis YaI, Pinchouk VG, Pinchouk LB.: Radiobiology aspects of accident on Chernobyl nuclear power plant. Kyiv, Naukova dumka, 1992 (in Russian). - 172 p.
17. Sidorenko SP, Berdova AG, Schlapatskaya LN.: Monoclonal antibodies of IPO series against B cell differentiation antigens in leukemia and lymphoma immunophenotyping. Neoplasma 1992; 39: 3-9.
18. Sun X-M, Snowden RT, Skilleter DN.: A flow cytometric method for the separation and quantitation of normal and apoptotic thymocytes. Anal. Biochem. 1992; 204: 351-354.
19. Uchara T, Miyawaki T, Ohta K.: Apoptotic cell death of CD45RO+ T lymphocytes in Epstein-Barr virus-induced infectious mononucleosis. Blood 1992; 78: 452-458.

Table 1
Number of cells (%) with bright staining of Hoechst 33342 and trypan blue dyeing in short-term cultures of blood mononuclears of examined persons

Object of investigation	Control cultures	Cultures with addition of inductors of apoptosis	
		IPO-4	Dex (5 mg/ml)
Blood mononuclears of healthy persons			
- Hoechst 33342 dyeing	14 ▪ 2	21 ▪ 9	20 ▪ 5
- Trypan blue dyeing	12 ▪ 2	15 ▪ 3	13 ▪ 5
Blood mononuclears of Chernobyl victims			
- Hoechst 33342 dyeing	15 ▪ 2	37 ▪ 5	49 ▪ 8
- Trypan blue dyeing	13 ▪ 2	21 ▪ 3	26 ▪ 4

Table 2
The subpopulation of peripheral blood mononuclears of examined persons, %

Antigen	Chernobyl victims	Healthy persons
CD5	54±7	60±3
CD4	41±3	50±2
CD8	22±2	24±2
CD45RA	44±2	54±4
CD45RO	30±3	30±2
HLA-DR	6±3	13±2
CD95	25±7	37±5
CD16	11±5	17±5
CD19	15±3	12±3