Apoptosis and Mutations in the Gene of T-cell Receptor of Blood Lymphocytes in Persons Chronically Exposed to Radiation

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INTRODUCTION

This study was focused on late effects of radiation exposure in members of the Techa River Cohort chronically exposed as a result of operations of the Mayak Production Association starting from 1949. The radiation exposure consisted of two components: external exposure to gamma radiation and internal exposure mainly to 90Sr, the major dose-forming radionuclide. The hematopoietic system is critical in this situation because of high radiosensitivity, and osteotropicity of 90Sr. The maximum dose rates to red bone marrow reached 0.77 Gy/year in 1951, and then they decreased exponentially down to the background levels. Late effects registered among Techa River cohort members included an increased risk of oncopathology (leukemia), elevated levels of chromosome aberrations and somatic mutations. These observations allow for an assumption that chronic exposure resulted in genome instability, which is manifested, among other things, by an increased level of somatic mutations.

The purpose of our research was to better understand the role played by apoptosis, the main defense-mechanisms of the cell (defense from the fixation of initial damage of genetic material in the form of mutations or/and aberrations). Also, the frequency of apoptosis and somatic mutations (CD3-CD4+) in lymphocytes of peripheral blood was estimated for residents of the Techa riverside villages who were chronically exposed to low and moderate doses.

METHODS

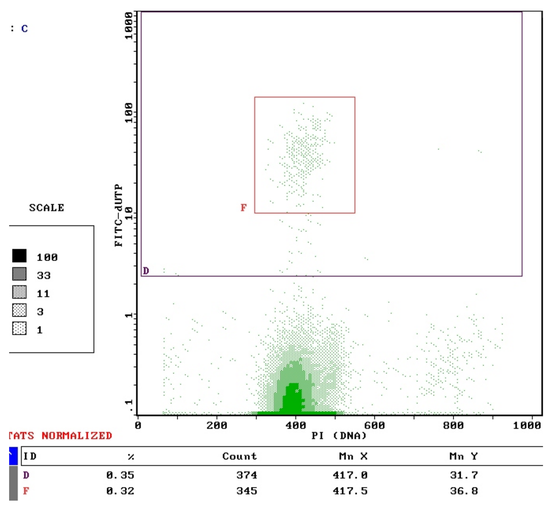

Fig.1 Apoptotic activity of peripheral blood lymphocytes was measured using the TUNEL methodology

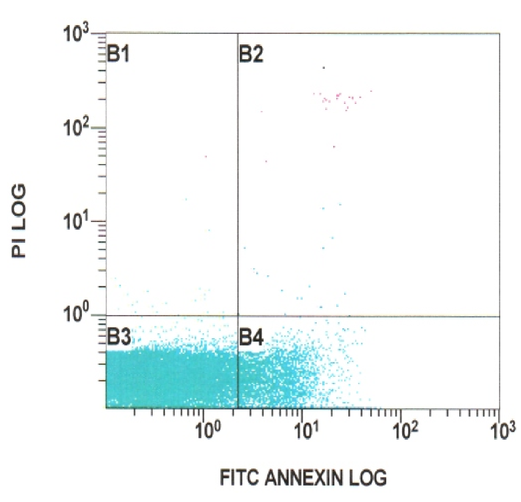

Fig.2 Apoptotic activity of peripheral blood lymphocytes was measured using the ANNEXIN V-FITC methodology

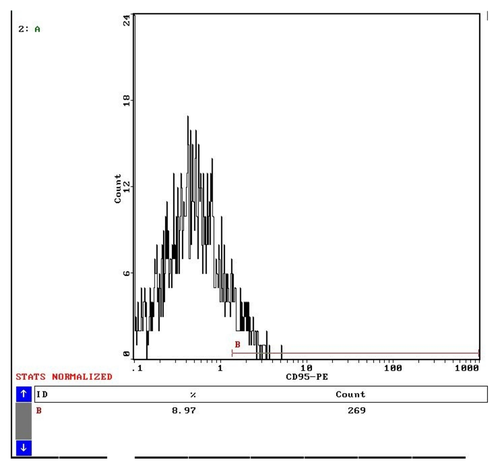

Fig.3 CD95 receptor on peripheral blood lymphocytes was measured using the CD typing methodology

CHARACTERISTICS OF THE GROUPS STUDIED AND RESULTS

Groups	Mean age, years M±m (min-max)	Gender				Ethnic composition			
		Male		Female		Slavs		Turkic	
		n	%	n	%	n	%	n	%
All exposed n=144	69.4±0.5 (58-83)	45	31.3	99	68.7	55	38.2	89	61.8
Exposed with CRS n=35	71.1±1.2 (60-83)	9	25.7	26	74.3	24	68.6	11	31.4
Control n=78	67.8±0.7 (56-81)	16	20.5	62	79.5	43	55.1	35	44.9
Note: n – number of examined individuals in group CRS - Chronic Radiation Syndrome									

Table 1. Characteristics of groups

Groups	Mean dose to RBM, Gy M±m (min-max)	Mean dose rate to RBM, Gy/year M±m (min-max)	Mean dose to whole body, Gy M±m (min-max)	Mean dose to whole body, mGy/year M±m (min-max)
All exposed n=144	1.05±0.06 (0.09-4.23)	0.26±0.02 (0.03-1.20)	0.064±0.01 (0.001-0.46)	26.0±4.0 (0.1-241.5)
Exposed with CRS n=35	1.00±0.1 (0.14-2.87)	0.22±0.03 (0.04-0.88)	0.053±0.02 (0.004-0.46)	23.0±9.0 (2.0±241.5)

Table 2. Dose characteristics of groups

Groups	Early apoptosis Median (25%-75%)	DNA fragmentation Median (25%-75%)	CD95$^+$cells Median (25%-75%)	Necrotic cells Median (25%-75%)	CD3-CD4+ cells Median (25%-75%)
All exposed	3.11 (1.24-5.36) p=0.01	0.15 (0.06-0.47)	2.05 (1.08-4.85)	0.01 (0-0.01)	0.33±0.08 (0.02-3.16) p=0.03
Exposed with CRS	2.37 (0.10-5.54)	0.16 (0.06-0.79)	1.96 (1.13-6.48)	0.01 (0-0.05)	0.40±0.16 (0.02-2.0) p=0.03
Control	0.52 (0.04-2.34)	0.11 (0.04-0.36)	2.51 (1.24-5.80)	0.01 (0-0.02)	0.11±0.02 (0.01-0.56)
Note: p - statistical significant differences from the control group.					

Table 3. The frequency of apoptosis, CD+cells, necrotic cells and CD3-CD4+ cells.

Fig. 4A

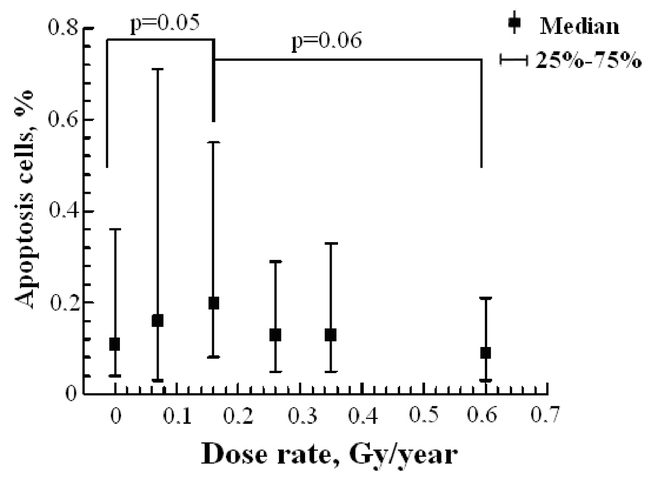

Fig. 4 B

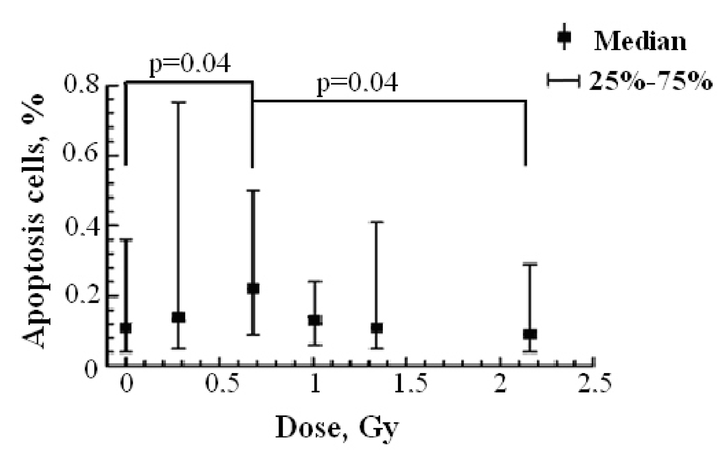

Fig. 4 Dependence of the apoptotic cell death with DNA fragmentation on the dose of RBM (A) and dose-rate irradiation RBM (B).

Groups	Baseline levels Median (25%-75%)	5 h incubation Median (25%-75%)	Irradiation 1 Gy + 5 h incubation Median (25%-75%)	24 h incubation Median (25%-75%)	Irradiation 1 Gy + 24 h incubation Median (25%-75%)
All exposed	3.11 (1.24-5.36) p=0.01	6.11 (3.87-10.42) p=0.001	5.21 (3.63-10.5) p=0.001	27.69 (16.52-35.0) p=0.005 p^*=0.001 p^{**}=0.002	26.16 (17.7-36.95) p^*=0.001 p^{**}=0.002
Exposed with CRS	2.73 (0.10-5.54)	3.79 (2.3-6.9)	2.94 (1.17-4.46)	36.85 (9.64-56.35) p^*=0.001 p^{**}=0.002	29.2 (0.43-40.6) p^*=0.001 p^{**}=0.002
Control	0.52 (0.04-2.34)	0.68 (0.36-0.86)	0.61 (0.38-1.30)	14.25 (4.62-18.2) p^*=0.001 p^{**}=0.001	18.35 (10.4-25.9) p^*=0.001 p^{**}=0.001
Note: p - statistical significant differences from the control group p^* - statistical significant intragroup differences from baseline levels of apoptosis p^{**} - statistical significant intragroup differences between 5 hours of incubation / 5 hours of incubation with pre-irradiation.					

Table 4. The frequency of apoptosis (%) under the additional load (early stage of apoptosis)

Fig. 5 A

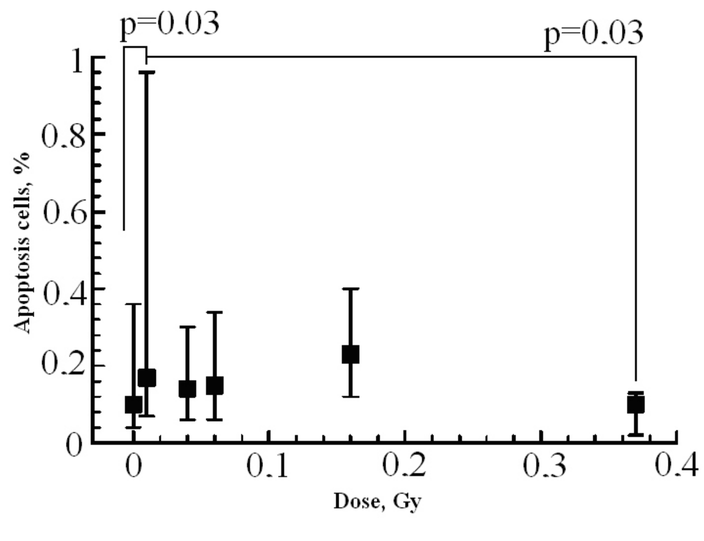

Fig. 5B

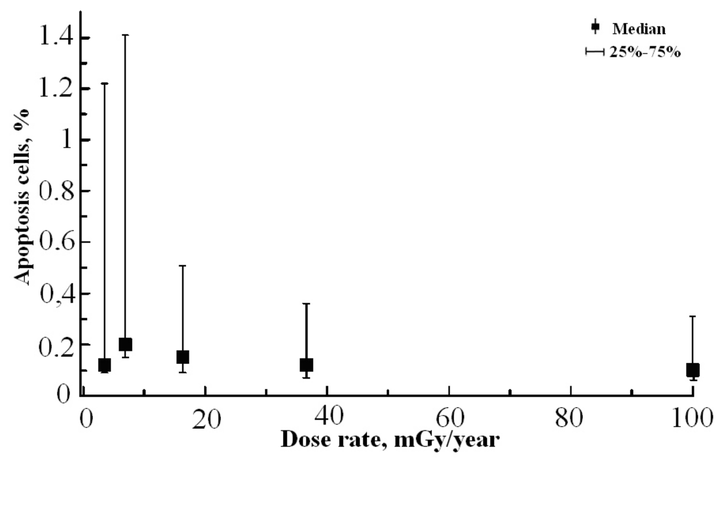

Fig. 5 Dependence of the apoptotic cell death with DNA fragmentation on the dose of whole body (A) and dose-rate irradiation Whole body (B).

Groups	Baseline levels Median (25%-75%)	5 h incubation Median (25%-75%)	Irradiation 1 Gy + 5 h incubation Median (25%-75%)	24 h incubation Median (25%-75%)	Irradiation 1 Gy + 24 h incubation Median (25%-75%)
All exposed	0.15 (0.06-0.47)	0.43 (0.12-0.86) p^*=0.01	0.53 (0.15-1.15) p^*=0.01	0.56 (0.24-1.73) p^*=0.01 p^{**}=0.04	0.69 (0.24-1.98) p^*=0.01 p^{**}=0.04
Exposed with CRS	0.16 (0.06-0.79)	0.50 (0.10-1.09)	0.72 (0.22-1.85) p^*=0.01	0.49 (0.14-2.30)	0.70 (0.18-1.45) p^*=0.01
Control	0.11 (0.04-0.36)	0.58 (0.19-1.24) p^*=0.01	0.54 (0.23-1.49) p^*=0.01	0.32 (0.08-1.72) p^*=0.01	0.76 (0.19-3.02) p^*=0.01
Note: p^* - statistical significant intragroup differences from baseline levels of apoptosis p^{**} - statistical significant intragroup differences between 5 hours of incubation / 5 hours of incubation with pre-irradiation.					

Table 5. The frequency of apoptosis (%) under the additional load (DNA fragmentation)

Factors	Baseline levels Median	24 h incubation	Irradiation 1 Gy + 24 h incubation
Nationality	p=0.4; F=0.47	p=0.7; F=0.1	p=0.9; F=0.02
Gender	p=0.5; F=0.46	p=0.3; F=0.16	p=0.2; F=1.7
Age	**p=0.05; F=2.76**	**p=0.001; F=8.83**	**p=0.02; F=3.45**
Dose to RBM	p=0.1; F=2.02	**p=0.001; F=6.41**	**p=0.05; F=2.53**
Dose to the whole body	**p=0.005; F=3.55**	p=0.4; F=1.7	p=0.2; F=1.52
Dose rate at the RBM	p=0.3; F=1.25	p=0.9; F=0.2	p=0.7; F=0.55
Dose rate at the whole body	**p=0.002; F=5.37**	p=0.9; F=0.15	p=0.9; F=0.16
Dose to RBM+Age	p=0.4; F=1.1	**p=0.001; F=7.39**	**p=0.004; F=4.02**
Dose to the whole body +Age	**p=0.001; F=3.17**	p=0.6; F=0.79	p=0.3; F=1.22
Note: F – coefficient of dispersion			

Table 6. The dependence of the frequency of apoptotic cell death (DNA fragmentation) by radiation and non-radiation factors.

CONCLUTION

The results of this research are another proof of the considerable role of apoptosis in the defense of the organism from accumulation of structurally and functionally defective cells, in particular under the conditions of chronic exposure. We have demonstrated that efficiency of apoptosis as the barrier mechanism on the way to genetically defective cell depends on the radiation dose. In the range of doses up to 0.2 Gy, apoptosis successfully compensates the increase of genetic damages. At doses above 0.2 Gy, there are cases when cells overcome the apoptotic barrier and the organism starts accumulating cells with somatic mutations. At doses above 0.8-1.0 Gy, arrest of apoptosis and impairment of its barrier function are observed.

At late time after the onset of exposure, residents of the Techa riverside villages manifested a 3-fold increase in the number of cells with T-cell receptor mutations in the exposed individuals and almost; a 4-fold increase, in the individuals with CRS. The number of apoptotic cells (Annexin) in the group of exposed individuals is higher with statistical significance compared to the control group.

This research has been conducted on peripheral blood lymphocytes and, therefore, our conclusions are related to the system of hematopoiesis; however, we assume that similar dependencies can be also observed in other proliferating tissues of the organism.

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