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Dynamics of Blood Cell Composition in Residents of the Techa Riverside Villages

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The present paper focused on the analysis of data resulting from 50-year studies involving assessment of the hemopoiesis state in 2,739 Techa riverside residents chronically exposed to radiation in the range from low to intermediate doses. The highest bone marrow doses (mean value: 0.66 Sv) were due to intake of ^{90}Sr in drinking water and local food products, and to external γ-exposure. The status of peripheral blood and bone marrow has been monitored since 1951. Peripheral blood cell counts performed for exposed riverside residents during the period of maximum radiation exposures (1951-1953) displayed a significant (p<0.0001) decrease in the proportion of leukocytes (neutrophils and lymphocytes) and thrombocytes. Peripheral blood erythrocyte count was maintained at the normal level, owing to the efficacy of compensatory processes in the bone marrow, including increased rates of erythrocaryocyte proliferation and maturation. The development of peripheral blood granulocytopenia resulted from delay in the differentiation of neutrophilic bone marrow granulocytes, a marked increase in the frequency of lethal abnormalities in bone marrow neutrophils, pathological mitoses, and activation of apoptosis. The time necessary for the blood cell composition to return to normal varied significantly in different blood cell series, depending on exposure dose-rate, extent of the initial hemopoiesis inhibition and individual physiological characteristics of exposed individuals. Thus, under a combined chronic exposure at equivalent doses to red bone marrow reaching 1.79 Sv, a stable reduction of neutrophil, thrombocyte and lymphocyte counts was observed. Neutrophilic granulocyte series were noted to require the longest time (12-17 years) to recover.

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Introduction

Radiation-induced inhibition of hemopoiesis often represents a critical phenomenon determining the extent of damage inflicted on the whole body by exposure to radiation. To date, both early and late effects of acute external exposure to γ-radiation have been studied in sufficient detail.[1-6] However, comparatively little attention was focused on initial reactions of the hemopoietic system and the hemopoiesis recovery processes in chronic (for multiple years) low dose-rate exposures in human. The latter studies were mostly based on the results from follow-up of atomic industry workers, i.e. healthy adult males who underwent selection procedures involving special medical examinations prior to employment.[8] Much less attention was given to the studies of the hemopoietic system in women, and also in persons exposed in childhood. Experimental studies in dogs have provided evidence of high plasticity of the hemopoietic system and its sufficient capacity to adapt to protracted γ-radiation, even in life-long exposures.[3,4]

Currently, it is well known that because of massive releases of radioactive waste from the radiochemical facility of the Mayak Production Association (MPA) into the Techa River in 1950-1956, residents of the riverside villages have been exposed to ionizing radiation for long years.[9] Because of the lack of reliable technologies for reprocessing and storing radioactive waste, beginning in March 1949 liquid radioactive wastes from the Mayak facility were discharged into the Techa River. Over the period from 1949 through 1952, 76 Mm^3 of sewage water with a total activity of about 3 MCi for ß-radiation was discharged into the Techa River. The discharges contained mixtures of the radionuclides ^{89}Sr, ^{90}Sr, ^{137}Cs, ^{95}Zr, ^{95}Nb, ^{103}Ru, ^{106}Ru and isotopes of rare-earth elements. Over a quarter of the total activity were contributed by the long-lived radionuclides ^{90}Sr and ^{137}Cs. Residents of the riverside settlements were chronically exposed to low dose-rate radiation, both external due to γ-radiation, and internal due to intakes of radionuclides with drinking water

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and local foodstuffs. Bone marrow (BM) is considered to be the critical organ in the population exposed on the Techa. Medical follow-up of the exposed population has been conducted since 1951, and it obligatorily includes complete blood counts, and, in a number of cases, BM studies (by medical indications). The results of long-term (for several decades) epidemiological studies are indicative of increased radiation risk of leukemia for residents of the Techa riverside villages.[10,11]

The purpose of our study was to assess the dynamics of the blood cell composition in Techa riverside residents during the period of radioactive waste discharge (1950-1956), and in the ensuing time period (up to 2001) which was characterized by gradual reduction in dose-rates and restoration of the background radiation to natural levels.

Subjects and Methods

The state of hemopoiesis in exposed persons was studied over the period from 1951 through 2001. The database at Urals Research Center for Radiation Medicine (URCRM) served as a key information source for investigating the changes in blood cell composition in residents of contaminated areas over a 50-year follow-up period. The study cohort comprised 2,739 persons who had lived in the contaminated riverside area since the time of the discharge into the Techa and had been undergoing medical examinations on regular basis. Patients diagnosed with diseases that could influence hemopoiesis parameters (tumors and hematological conditions) were not included in the study. Also, excluded from the analyses were data on the blood parameters measured in patients during acute phases or exacerbations of their chronic inflammatory diseases. The study group included 1,139 exposed men (41.6%) and 1,600 exposed women (58.4%). As of the start of exposure, the age of the study group members ranged from 1 to 67 years with the mean (standard deviation or SD) of 21.7 (14.4) years, and by the end of the follow-up (1995-2001) they reached from 44 to 80 years with the mean (SD) of 61.6 (7.5) years.

BM doses were estimated on the basis of the Techa River dosimetry system 2000 (TRDS-2000).[7] Maximum dose-rates, reaching 0.29 Sv/yr, were estimated for the years 1950 and 1951. The dynamics of exposure dose-rates showed a tendency towards reduction, especially after 1956, i.e. by the time massive discharges of radionuclides into the river system had been stopped. BM doses had mostly accumulated by 1960 and the cumulative BM doses reached the maximum of 1.79 Sv.

The control group was composed of people living in the same villages as members of the study group. BM doses received by control individuals did not exceed 5 mSv/yr during the period of the maximum radiation exposure. The study and the control groups were comparable in terms of proportions of people representing different age categories and genders. The control group consisted of 306 persons, including 128 (41.8 %) men and 178 (58.2%) women. As of 1950, the age of the control group members ranged from 1 to 64 years with the mean (SD) of 23.3 (12.5), and by the end of the follow-up (1995-2001), they reached 44-80 years with the mean (SD) of 59.6 (9.2) years. It should be noted that the analysis was based on the hematological data resulting from medical examinations of persons aged 16 and older.

Cytological studies of bone marrow were performed in 44 adult Techa riverside residents aged 16-48 years with the mean (SD) of 24.6 (10.2) years. BM biopsy specimens obtained in 1954-1956 with sternal puncture were reviewed retrospectively. Male patients accounted for 50% of the total examined subjects. The control group included 16 unexposed individuals aged 16-49 years with the mean (SD) of 24.8 (9.2) years, and 5 of them underwent thorough medical examinations since they were bone marrow donors, and examinations for presumed hematological conditions in the remaining 11 produced negative results.

Throughout the follow-up period, hematological studies were performed using standard laboratory diagnostic methods.[12,13] The status of the BM hemopoiesis was studied by scoring standard myelograms (scoring 200 cells), partial erythro- and granulocytograms[14] (scoring 1,000 cells per each cell series). The hematological studies focused on BM cell abnormalities (binuclear and giant cells, fragmentation of nuclei, caryolysis, pycnosis of nuclei, micronuclei and cytoplasmic bridges), defective mitoses (attached metaphases and interchromosomal bridges) and proliferative activity of the erythrocaryocytes and neutrophilic granulocytes.

Comparison of respective continuous parameters between the exposed and control groups was performed using Student's *t*-test in case of normally distributed parameters, and Mann-Whitney *U*-test in other cases. Comparison of the frequency between the exposed and control groups was based on Fisher's exact test. Necessary calculations were performed using SPSS ver. 10 for Windows (SPSS Inc., Chicago, IL).

Results

Bone marrow hemopoiesis during the period of maximum exposures

It was established that BM cellularity, relative proportion of neutrophil granulocytes and leuko-erythroblastic ratio observed in exposed persons and controls over the period 1954-1956 were comparable. The number of myelocaryocytes in the exposed group was in the range of 42.5-340.5 $\times 10^9$/L with the mean±SD of 132.0±72.7 $\times 10^9$/L, and that in the control group was in the range of 44-189 $\times 10^9$/L with the mean±SD of 102.3±42.3 $\times 10^9$/L. The relative proportion of BM neutrophil granulocytes was 66.6±7.3% and 68.5±6.8% in the study and control groups, respectively. The proportion of BM erythrocaryocytes was significantly increased (p=0.026) in the exposed group (24.1±7.6%) compared to control group (20.5±5.4%). Leuko-erythroblastic ratio did not differ significantly between the study group (3.6) and the control group (4.2).

Conventional analyses of myelograms (Table 1) indicated a significant decrease only in the number of erythroblasts, and a significant increase only in oxyphilic normocytes in the exposed group

Table 1. Distribution of bone marrow cell composition in exposed and control groups in 1954-1956

Parameter	Exposed group	Control group	*p*-value[a]
Undifferentiated blasts (%)	0.1±0.33[b]	0.03±0.12	0.2354
Myeloblasts (%)	0.7±0.8	0.5±0.5	0.4866
Promyelocytes (%)	1.2±1.0	0.8±0.5	0.2797
Neutrophilic myelocytes (%)	18.0±4.4	17.1±5.1	0.4820
Metamyelocytes (%)	15.9±3.7	17.5±4.6	0.2469
Band neutrophils (%)	17.9±4.2	18.0±4.4	0.9930
Segmented neutrophils (%)	12.9±5.1	15.8±4.9	0.0528
Neutrophil maturation index	1.2±0.4	1.1±0.5	0.5463
Total eosinophils (%)	3.7±2.2	3.1±1.9	0.3574
Total monocytes (%)	0.07±0.17	0.2±0.4	0.2061
Lymphocytes (%)	4.2±3.5	6.5±3.7	0.0516
Plasmocytes (%)	1.0±0.8	1.0±0.9	0.9740
Erythroblasts (%)	0.08±0.2	0.3±0.3	0.0055
Pronormoblasts (%)	1.2±0.9	0.8±0.7	0.1853
Basophilic normocytes (%)	3.6±2.0	3.3±2.1	0.8000
Polychromatophlic normocytes (%)	8.5±4.5	8.3±4.0	0.8272
Oxyphilic normocytes (%)	10.8±3.9	6.8±3.5	0.0003

[a]Based on Student's *t*-test comparing the exposed and control groups.
[b]Mean±standard deviation.

Table 2. Distribution of proliferative activity of erythrocaryocytes in exposed and control groups in 1954-1956

Parameter	Exposed group	Control group	*p*-value[a]
Mitoses, per 1,000 erythrocaryocytes	18.3±5.7[b] (7-33)[c]	14.3±3.4 (7-19)	0.0028
Mitotic index of erythroblasts and proerythroblasts (%)	8.3±4.9 (0-20)	7.2±4.9 (0-18.5)	0.2599
Mitotic index of basophilic normocytes (%)	9.6±5.1 (1.6-23.1)	6.1±2.9 (0-9.9)	0.0013
Mitotic index of polychromatophilic normocytes (%)	1.3±1.1 (0-4.2)	0.8±0.4 (0-1.7)	0.0152

[a]Based on Student's *t*-test comparing the exposed and control groups.
[b]Mean±standard deviation.
[c]Minimum-maximum

compared to the control group. No significant difference was observed between the exposed and control groups in the myeloid cell series as well as in lymphocyte, monocyte and plasmocyte counts. Besides, there was no significant difference in the neutrophil maturation index (MI) between the exposed and control groups.

Normal myelographic findings were observed during the period of maximum radiation exposure in 31.2 % of the control group, only in 2.3% of the exposed group. Residents of the Techa riverside villages manifested a significantly (p=0.019) higher frequency (36.4%) of erythrocytic series hyperplasia (erythrocaryocytes > 26.5%) than the controls (6.2%).

Studies of proliferative activity of BM neutrophils and mitotic index of neutrophilic myelocytes did not reveal any difference between the exposed and control groups. The frequency (±SD) of mitoses per 1,000 neutrophilic BM granulocytes was 3.4±2.5 and 3.7±1.2 in the exposed and control groups, respectively; the mitotic index (±SD) of myelocytes in the respective groups was 1.1±0.8% and 1.4±0.6%.

The differentiation of neutrophilic BM granulocytes in the two groups was assessed using the method of partial granulocytograms. During the period of maximum radiation exposures an increased production of myeloblasts (1.04% in exposed individuals *vs* 0.54% in controls, p=0.0051) and neutrophilic myelocytes (28.0% in exposed individuals *vs* 24.3% in controls, p=0.0091) was observed. This fact indicates a delay in granulocyte maturation in individuals chronically exposed to radiation.

Proliferative activity of erythrocaryocytes was significantly higher in the exposed group than in the control group (Table 2). The mitotic indices of basophilic normocytes and polychromatophilic normocytes were both higher in the exposed group than in the control group. Individuals with increased mitotic activity of erythrocaryocytes were seen significantly (p=0.0126) more frequently in the exposed group

(31.8%) than in the control group (0%).

It was established that in 1954-1956 the differentiation of erythrocaryocytes had undergone certain changes in the exposed group. A statistically significant increase in oxyphilic normocytes (51.4% in the exposed group *vs* 42.4/% in the control group; p= 0.0013) was observed along with a statistically significant decrease in polychromatophilic normocytes (33.6% in the exposed group *vs* 39.7% in the control group; p=0.017). In addition, a statistically significant reduction in erythroblasts was registered (0.6% in the exposed group *vs* 1.1% in the control group; p=0.0186).

Assessment of the erythropoiesis status made it possible to identify a significant number of chronically exposed persons (31.8%) in whom increased proliferation of erythroid cells was combined with increased production of mature erythrocaryocytes (cells at oxyphilic normocytes phase > 5.6%). Besides, 35.7% of them were noted to have developed hyperplasia of bone marrow erythrocytic series (erythrocaryocytes in the bone marrow aspirate > 26.5%).

Frequency of deficient mitoses and abnormalities in BM hemopoietic cells at the interphase stage

The frequency of pathological mitoses in myeloid and erythroid cell series (mostly "attached metaphases") and lethal abnormalities in BM hemopoietic cells registered in 1954-1956 was significantly higher in the exposed group than in control group (Figure 1). Deficient mitosis was significantly more frequent in the exposed group than in the control group, especially in the erythroid cell series. Bridges between chromosomes were observed very infrequently and only in cells of the erythroid series at annual dose-rate exceeding 0.1 Sv.

The results from the studies of abnormalities detected at the interphase stage in BM neutrophils are exhibited in Table 3. Aberrant neutrophils were significantly more frequent in the exposed group than in the control group. Attention is drawn to a significantly increased number of cells with lethal aberrations in exposed group during the period of maximum radiation exposure. Compared to the

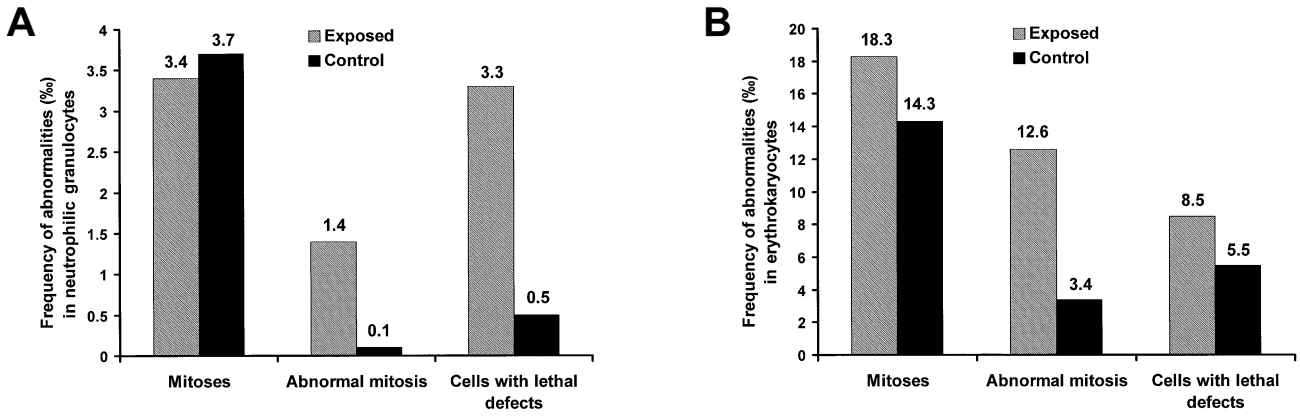

Figure 1. Frequency of mitoses, defective mitoses and lethal abnormalities in red bone marrow cells of chronically exposed individuals for the period of 1954-1956. **A.** The neutrophilic series of chronically exposed individuals exhibited a significant increase in the frequency of defective mitoses (attached metaphases) (p=0.0054, Mann-Whiney U-test) and of interphase cells with lethal aberrations (p<0.0001, Mann-Whiney U-test) compared to the controls. However, no significant difference was observed between the exposed and control groups in the frequency of mitoses in neutrophilic granulocytes. **B.** The frequency of defective mitoses and cells with lethal abnormalities detected in the erythrocytic series of exposed individuals was also found to be significantly higher than that in controls (p<0.0001, Mann-Whiney U-test and p=0.0105, Student's t-test, respectively). The frequency of mitoses in erythropoietic cells was also significantly higher in exposed individuals than in controls (p=0.0280, Student's t-test).

Table 3. Frequency of abnormalities per 1,000 bone marrow neutrophilic cells at interphase in exposed and control groups in 1954-1956

Abnormalities	Exposed group	Control group	p-value[a]
Binuclear cells	0.3±0.6[b] (0-2)[c]	0.2±0.4 (0-1)	0.4931
Giant cells	0.5±1.3 (0-7)	0.1±0.3 (0-1)	0.5418
Fragmentation of nuclei	2.1±3.3 (0-13)	0.3±0.8 (0-3)	0.0021
Caryolysis	0.5±2.0 (0-10)	0	0.5037
Total lethal abnormalities	3.3±3.7 (0-13)	0.5±0.8 (0-3)	0.0001
Pycnosis of nuclei	6.4±10.9 (0-47)	0.4±1,0 (0-4)	0.0002
Total abnormalities	9.8±12.8 (0-57)	0.9±1.1 (0-4)	0.0002

[a]Based on Mann-Whitney U-test comparing the exposed and control groups.
[b]Mean±standard deviation.
[c]Minimum-maximum.

control group, the exposed group showed more frequently such signs of apoptosis as pycnosis, fragmentation of nuclei and caryolysis in neutrophilic granulocytes of the bone marrow. Cells with caryolysis were observed only in BM neutrophilic series in the exposed group.

It was noted that during the maximum exposures the BM erythrocytic series in the exposed group contained a considerably larger number of abnormal cells and cells with lethal defects than those in the control group (Table 4). Of special importance was high frequency of erythrocaryocytes linked by cytoplasmatic bridges.

Peripheral blood cell composition during the period of maximum radiation exposure (1951-1956)

It should be noted that during the maximum radiation exposure the changes going on in the bone marrow were reflected in the peripheral blood cell composition. Blood leukocyte and thrombocyte counts were highly dependent on BM dose-rates. As is evident from Table 5, the peripheral blood of the chronically exposed group was characterized by decreased counts of leukocytes and their main population (lymphocytes and neutrophils), and thrombocytes. The lowest values of these parameters were observed during the first 3 years of exposure (1951-1953) when the highest BM dose-rates were registered. During the same time interval, the exposed group manifested decreased frequency of peripheral blood monocytes and basophils. The count of erythrocytes was within the normal limits in the exposed group for both genders and did not differ significantly from that in the control group. The stability of peripheral blood erythrocyte counts in the exposed group was probably maintained from 1951 to 1956 owing to adequate compensatory processes in the bone marrow.

Table 4. Frequency of abnormalities per 1,000 erythrocaryocytes in exposed and control groups in 1954-1956

Abnormalities	Exposed group	Control group	*p*-value[a]
Interchromosomal bridges	0.2±0.4[b] (0-2)[c]	0	0.5777
Binuclear cells	5.9±3.4 (0-15)	4.7±2.5 (1-11)	0.1835
Micronuclei	2.6±2.1 (0-8)	0.8±0.8 (0-3)	0.0003
Total lethal abnormalities	8.5±4.3 (0-20)	5.5±2.7 (1-12)	0.0105
Cytoplasmic bridges	20.3±12.0 (3-47)	3.8±2.9 (1-10)	<0.0001
Pycnosis of nuclei	0.13±0.4 (0-2)	0	0.7850
Total abnormalities	30.0±12.9 (0-62)	9.1±3.4 (4-16)	<0.0001

[a]Based on Student's *t*-test comparing the exposed and control groups except for interchromosomal bridges, pycnosis of nuclei and micronuclei for wich Mann-Whitney *U*-test was used.
[b]Mean±standard deviation.
[c]Minimum-maximum.

Table 5. Distribution of peripheral blood cell composition in exposed and control groups in the period of maximum radiation exposure (1951-1956)

Parameter	Exposed group		Control group	*p*-value[a]	
	1951-1953	1954-1956		1951-1953	1954-1956
Leukocytes ($\times 10^9$/L)	5.3±1.4[b]	5.7±1.5	6.4±1.4	<0.0001	<0.0001
Thrombocytes ($\times 10^9$/L)	190.4±42.8	223.3±63.9	256.1±65.3	<0.0001	<0.0001
Neutrophils ($\times 10^9$/L)	3.0±1.0	3.1±1.2	3.5±1.1	<0.0001	<0.0001
Lymphocytes ($\times 10^9$/L)	1.8±0.6	2.0±0.7	2.2±0.8	<0.0001	0.0594
Stab neutrophils (%)	8.0±5.2	8.9±5.8	7.3±4.4	0.0927	<0.0001
Segmented neutrophils (%)	48.3±9.0	43.6±11.4	46.4±10.5	0.0505	0.0002
Erythrocytes ($\times 10^9$/L)					
Male	4.3±0.5	4.5±0.5	4.6±0.4	0.1110	0.1693
Female	4.0±0.4	4.2±0.5	4.2±0.4	0.0516	0.1278
Monocytes (%)	6.3±2.8	7.6±3.7	7.3±3.7	0.0002	0.1356
Eosinophils (%)	3.6±2.7	3.2±2.6	3.7±2.6	0.5023	0.1170
Basophils (%)	0.18±0.47	0.37±0.68	0.28±0.64	0.0113	0.0235

[a]Based on Student's *t*-test comparing the exposed and control groups in the respective periods.
[b]Mean±standard deviation.

Decreased counts of leukocytes noted over the initial 3 years of radiation exposure were associated with decreased counts of both lymphocytes and neutrophils, and in the ensuing years only with decreased levels of neutrophils. In 1954-1956 an increase in stab neutrophils was noted in differential leukocyte count.

In the exposed group a significant (p<0.0001) increase in the frequency of moderate leucopenia (leukocyte counts being from 3.3×10^9/L to 4.0×10^9/L) was observed during the period of maximum exposures. It was registered in 11.1% of the exposed group 1-3 years after the start of exposure, and diminished to 7.3% in 1954-1956. A marked decrease in blood leukocytes (< 3.3×10^9/L) registered in 2.6% of the exposed group from 1951 to 1953 decreased to 1.5% in 1954-1956. One per cent of the control group manifested only moderate leucopenia.

Over the initial 6 years of chronic radiation exposure, a reduction in the average leukocyte counts in the exposed group was attributed to an increased number of individulas with leucopenia, and to the fact that leukocyte counts were approaching the lower limit of the physiological norm. The distribution of leukocyte counts in the exposed group depicted markedly asymmetric curves for the years 1951-1953 and 1954-1956, with a shift towards lower leukocyte counts compared to the control group (Figure 2).

A significant (p=0.0205) increase in the frequency of lymphopenia in the exposed group compared to the control group was only observed during the initial 3 years after the start of chronic exposure, without any increase in the frequency of manifest lymphopenia (lymphocyte <0.8×10^9/L). Neutropenia was significantly (p=0.0004) more frequently observed in the exposed group than in the control group 4-6 years after the start of exposure: in 1.6% and 13.8% of the exposed group, neutrophil counts were below 1.3×10^9/L (pronounced neutropenia) and were within the range of 1.3-1.9 $\times10^9$/L (moderate neutropenia), respectively. In the control group, only 7.2% individuals were noted to have developed moderate neutropenia.

Thrombocytes in the exposed group were significantly reduced in 1951-1956, especially during the first 3 years compared to the control group (Table 5). Those with thrombocyte counts in the range of 150-180 $\times10^9$/L (moderate thrombocytopenia) and those with the counts below 150×10^9/L (pronounced thrombocytopenia) were significantly more frequent in the exposed group than in the control group (p<0.0001 and p=0.0003, respectively). Besides, with a decrease in exposure dose-rate to BM overtime, was decreasing the proportion of individuals with moderate thrombocytopenia (27.6% in 1951-1953 and 13.7% in 1954-1956 in the exposed group, and 4.7% in the control group), and pronounced thrombocytopenia (14.4% in 1951-1953 and 10.1% in 1954-1956 in the exposed group, and 4.1% in the control group).

As seen from Figure 3, the distribution of thrombocytes in the exposed group in 1951-1953 and 1954-1956 shifted to the left from that in the control group, and the shift was most pronounced in 1951-1953.

Erythrocytes in the exposed group were normal in both genders on the whole during the initial 6 years of exposure, and did not differ from those registered in the control group. Frequency of individuals with reduced erythrocyte counts (erythropenia) and that of those with increased counts (erythrocytosis) were both similar in exposed and control groups.

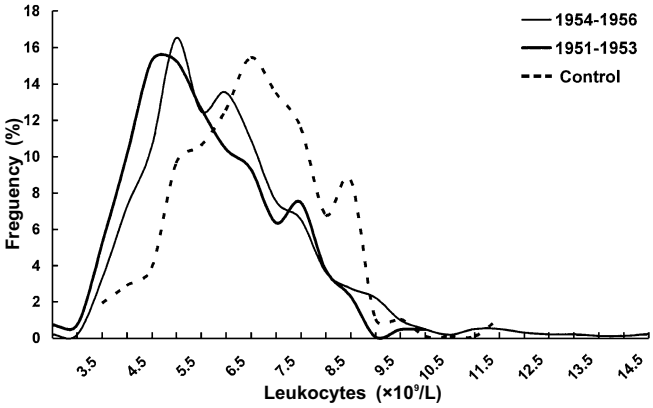

Figure 2. Distribution of individual peripheral blood leukocyte counts in residents of the Techa River villages (solid line) and controls (dashed line) for the period of 1951-1956.

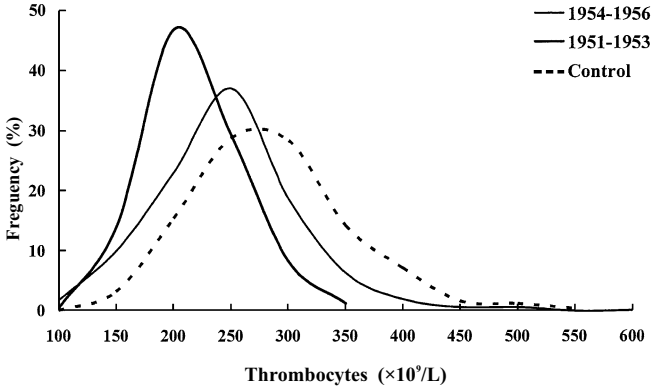

Figure 3. Distribution of individual peripheral blood thrombocyte counts in exposed individuals (solid line) and controls (dashed line) for the period of 1951-1956.

Dynamics of blood cell composition in persons chronically exposed to radiation in 1951-2001

Throughout the follow-up period, erythrocyte counts in men and women of the exposed group were maintained at a normally stable level. Leukocyte, neutrophil, lymphocyte and thrombocyte counts depended on BM dose-rate. As indicated in the previous section, the lowest counts of leukocyte, neutrophil, lymphocyte and thrombocyte were observed during the period of maximum radiation exposure, while after the dose-rates to BM had decreased, a marked tendency towards normalization of the counts of these cells became apparent as is demonstrated by the linear trend in Figure 4.

The most significant reduction in leukocyte counts was observed during the first decade after the start of exposure (Figure 4 A). The normalization of leukocyte count proved to be a lengthy process.

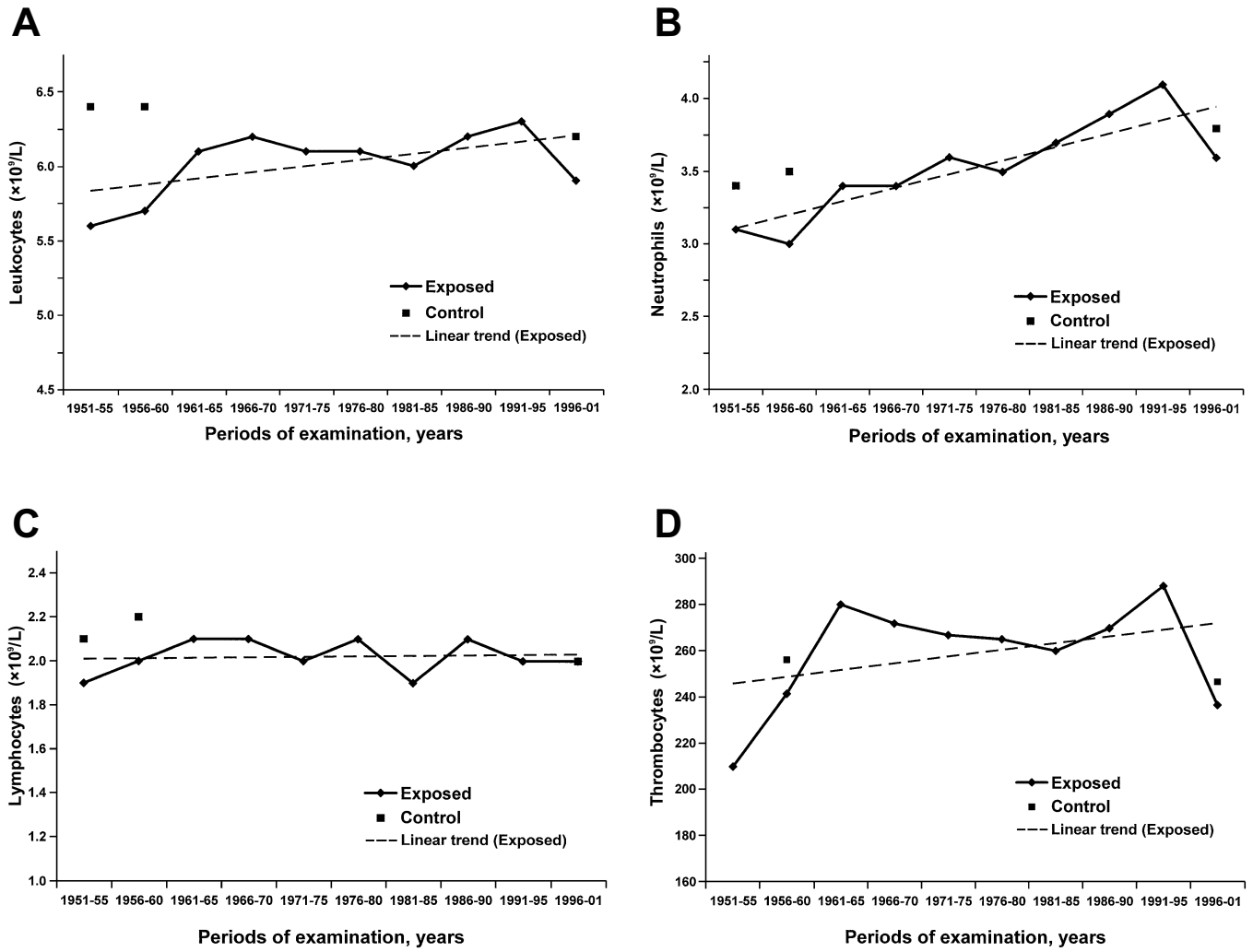

Figure 4. Dynamics of peripheral blood cell counts in individuals chronically exposed to radiation in the Techa riverside villages over 50 years of follow-up. In comparison to controls, exposed individuals manifested decreased leukocyte counts (**A**) during the following periods of follow-up: 1951-1965 ($p<0.0001$), 1966-1970. ($p=0.0286$), 1971-1975 ($p=0.0011$), 1976-1980 ($p=0.0004$), 1981-1985 ($p<0.0001$); neutrophil counts (**B**) were found to be decreased in 1951-1960 ($p<0.0001$). Decreased lymphocyte counts (**C**) were noted in exposed individuals compared to controls in 1951-1960 ($p<0.0001$), 1971-1975 ($p=0.0005$) and 1981-1985 ($p<0.0001$). Thrombocyte counts (**D**) were reduced in 1951-1955 ($p<0.0001$) and 1956-1960 ($p=0.0003$). The dashed line indicates a linear trend for exposed individuals. The mean cell counts in control individuals relate to the initial (1951-1960) and the closing (1996-2001) periods of the follow-up. All of the *p*-values were based on Student's *t*-test.

Leukocyte counts observed over 35 years after the start of exposure were significantly lower in the exposed group than in the control group. Since 1986, no statistically significant difference has been observed in the average leukocyte counts between the exposed and control groups. Over the entire follow-up period, the proportion of individuals with moderate leucopenia was higher in the exposed group (4-12%) than in the control group (1.0-1.2%). Individuals with manifest leucopenia were registered in the exposed group over the period from 1986 to 1990, while no such cases were seen in the control group. In the late period after the start of chronic radiation exposure (50 years later) only a few individuals with BM doses reaching 1.79 Sv (mean dose of 0.66 Sv) had marked peripheral blood leucopenia.

Reduction in neutrophil counts persisted over the first 10 years of exposure, which caused a decrease in leukocyte counts (Figure 4 B). The minimum level of neutrophilic granulocytes was noted in 1956-1960. Besides, a decrease was noted in the composition of the leukocyte population due to an increase in stab cells, the phenomenon being most manifest in 1954-1956. From 1960 onward, the level of neutrophils kept increasing; however, no difference from the values registered in the control group was traced. The proportion of individuals with neutropenia was highest over the first 10 years of exposure, reaching 16% in 1960, the 10th year of exposure. From 1976 onward, the incidence of neutropenia (neutrophil $<2\times10^9$/L) observed in 1.1-5.3% of the exposed group did not exceed that seen in the control group (6%).

The minimum level of lymphocytes relative to the control group was observed during the initial 5 years of exposure when the highest

BM dose-rates were observed (Figure 4 C). In the ensuing years, no clear-cut dynamics was noted in lymphocyte counts. Although the incidence of lymphopenia registered during the overall follow-up period in the exposed group did not, as a rule, exceed that in control group, the mean counts of lymphocytes in the exposed group were found to be periodically lower than those in the control group.

The dynamics of thrombocyte counts depended on dose rate of BM exposure (Figure 4 D). The maximum decrease in the thrombocyte counts as well as in the counts of other blood cells was registered over the period characterized by the highest dose-rates. The level of thrombocytes was restored to normal 10 years after the start of exposure, and it was associated with a significant reduction in the dose-rate of BM exposure. From 1961 onward, the mean levels of these cells in the exposed group did not differ significantly from the respective mean levels in the control group, and the incidence of thrombocypenia, registered in 1956 and later in the exposed group did not exceed that in the control group for all time periods.

Discussion

Long-term exposure of the bone marrow to low dose-rate ionizing radiation, including external exposure to γ-radiation and intake of ^{90}Sr through drinking water and local foodstuffs, considerably affected hemopoiesis in residents of the Techa riverside villages. The most pronounced changes in the hemopoietic system were observed in 1951-1953 when the dose-rate of BM exposure reached 0.29 Sv/yr. At that time, decreased counts of neutrophilic granulocytes, lymphocytes and thrombocytes were noted in a significant number of individuals of the exposed group. As a result of reduction in the exposure dose-rate (not exceeding 0.11 Sv/yr) in 1954-1956, leukocyte counts showed a tendency towards normalization.

The changes observed in the peripheral blood cell composition reflected the reactions of different BM series to chronic radiation exposure. The stability of normal erythrocyte counts registered in residents of the Techa riverside settlements was maintained owing to adequate compensatory changes in the erythroid series of the bone marrow: intensified proliferation rate (at the phases of basophilic normocyte and polychromatophlic normocyte) and increased rate of erythrocaryocyte differentiation. Hyperplasia of the erythroid cell series observed during the period of maximum radiation exposure indicates expanded potential of erythpoiesis. Comparison of the number of cells between those undergoing mitosis and those with lethal damage showed that the proportion of erythrocaryocytes with lethal aberrations in the erythroid series in the exposed group (8.5‰) was more than twice as low as that of dividing erythrocaryocytes (18.3‰). The situation in the control group was similar: the number of mitoses was essentially higher than that of cells with aberrations. The above-mentioned data suggest that in chronic exposure (BM dose-rate $\leq$0.6 Sv/yr) radiation-induced lethal damage to erythrocaryocytes is compensated for by intensified proliferative activity in the pool of proliferating-maturing cells of the erythroid series and by their accelerated maturation, ensuring the numerical stability of erythrocytes in the peripheral blood.

On the other hand, the state of the pool of proliferating-maturing BM neutrophils during the period of maximal exposure demonstrated that the alteration process prevailed over repopulation reflected in the peripheral blood cell composition. Reduction in the leukocyte counts to 2.2×10^9/L and that in neutrophils to 0.9×10^9/L observed in some of the chronically exposed individuals can be attributed to inefficiency of the compensatory-recovery processes in the bone marrow granulocytopoiesis. Thus, the mean number of cells with lethal defects identified in the neutrophilic series (3.3‰) was comparable to that of cells undergoing mitotic processes (3.4‰), while the level of bone marrow neutrophils in the state of mitosis observed in the control group (3.7‰) was much higher than that of cells with lethal aberrations (0.5‰).

Another factor bringing about deficiency of segmented neutrophils in the peripheral blood is a delay in maturation of neutrophilic granulocytes in the bone marrow (primarily at the myelocytic stage). It was revealed based on the findings of myelopoiesis studies performed for a number of exposed individuals that the number of mature neutrophils persisted as reduced both in bone marrow and in blood despite the increased proliferation of neutrophilic granulocytes in bone marrow. At the same time, the neutrophil maturation index was found to be increased. We may infer, consequently, that the mechanisms responsible for cell loss compensation were not effective enough to lead to adequate repopulation of BM neutrophils, which prevented a sufficient number of these cells from entering the blood flow. Thus, for a long time period, granulocytopoiesis failed in adequate compensation for cell loss resulting from radiation exposure and physiological regeneration in exposed individuals.

The studies of the exposed individuals made it possible to show that during the period of maximum exposures a significant proportion of dividing BM neutrophils and erythrocaryocytes manifested morphological signs of abnormal mitotic processes (chromosomal stickiness), a phenomenon that was reflected in an increased frequency of "linked metaphases." However, the role played by abnormalities of BM cell mitosis in the induction of post-radiation hemopoiesis inhibition has not been studied in sufficient detail so far.

It should be noted that in the framework of the present study we have not investigated the repair processes of radiation-induced cell damage, reaction to exposure of the pluripotent stem cells and committed progenitors, which, according to experimental data, are of great importance for repopulation of hemopoietic cells after chronic exposure.[2,3,6,18] Experiments[6] using rats of Wistar line, and mice of BALB/c and CBA lines exposed to long-term irradiation (exposing radioresistant CBA line mice to γ-radiation at doses of 0.01 to 0,5 Gy/day with cumulative doses amounting to 2-30 Gy, and BALB/c line rats and mice at doses of 0.25-0.5 Gy/day) showed that the earliest and the most intensive was depopulation of the stem cell compartment (CFU-S). The depletion of the pools of committed progenitors of erythropoietic and granulocytic cells occurred at a later time, the rate of depletion being lower compared to those noted for polypotent CFU-S. Long-term external exposure to γ-

radiation brings about depletion of the stem cell compartment parallel with increase in the proliferative activity of the cells. Intensified proliferative activity of polypotent CFU-S can be registered at exposure doses of 0.2-0.3 Gy. Stabilization of the CFU-C population is followed by increase in the proportion of committed progenitors. It should be noted that committed progenitors are capable of proliferation too. It was established that under continuous irradiation the rate of restoration of the stem cell pool depends on dose-rate. Normalization of proliferating, maturing and functional pools, as well as of CFU-S population, was going on slower after irradiation at lower daily-dose than that seen at higher dose-rate, but at compatible total dose.

Restoration of the composition of hemopoietic cell series occurred within different time periods after the start of radiation exposure. Lymphocyte count was the first to return to normal. Thus, from 1961 onward, the mean counts of lymphocytes and thrombocytes, as well as the number of individuals with lymphopenia and thrombocytopenia, did not differ from the respective characteristics obtained in the control group. Afterward neutrophil count became normal. The longest time was required for the entire population of circulating leukocytes to recover: 8% of the exposed individuals manifested reduced functional activity of the leukocytic series late after the start of exposure (50 years later). The data from the study thus point out a long duration of the leukocyte count recovery process under chronic radiation exposure of BM dose reaching 1.79 Sv.

The duration and completeness of normalization of leukocyte count were to a significant degree determined by the extent of hemopoietic function inhibition during the period of maximum radiation exposure. The longest recovery period and increased frequency of leukopenia was observed late in persons who had shown marked hemopoiesis inhibition during the initial period of exposure. It should be noted that individual characteristics such as gender and age at the time of exposure exert significant modifying effects on the recovery of leukocyte count in chronic exposure. In women the time for leukocyte count to recover was longer than that in men. The hemopoietic system in younger persons (up to 20 years) was noted to have higher capacity to recover leukocyte count than older persons exposed to similar BM dose.

As was revealed by our studies, the factors influencing the dynamics of changes in the blood cell composition observed in the exposed residents of the Techa riverside area are compatible to those noted in Mayak Production Association workers exposed mostly to external γ-radiation for years.[15-17] It has been shown that exposure at dose-rate of 0.25-0.5 Gy/yr and total dose of 1.5-2.0 Gy result in induction of unstable thrombocytopenia and leucopenia in able-bodied men, and at annual dose exceeding 0.5 Gy the reduction in thrombocyte and leukocyte (mostly neutrophilic granulocytes) count assumes a persistent character. After the termination of exposure, individuals with total dose of 2-9.33 Gy (annual dose >1.0 Gy) exhibited gradual normalization of peripheral blood cell counts. However, 35-40 years later, 20% of them still manifested moderate peripheral blood leucopenia, 7.3% showed moderate bone marrow hypoplasia, and 4.3% partial hypoplasia of granulocytopoiesis. No manifest dependency on exposure dose was traced in the frequency and intensity of leucopenia 40 years after the start of exposure. No dose dependency was traced in the frequency of bone marrow hypoplasia; it was noted, however, that most of the individuals displaying granulopoiesis hypoplasia later had been exposed to ^{239}Pu.

Thus, chronic exposure, even of low dose-rate, but for a prolonged time period, may bring about deficiency of compensatory-adaptive mechanisms for inadequacy of the adaptive capacity of the hemopoietic system.[3,4,8] Under the conditions of predominating alteration processes in chronic exposure, accumulation of radiation-induced damage is going on in hemopoietic cells. Physiological loss of mature cells taking place in normally functioning organism may be inadequately compensated for under chronic exposure by increased production of these cells because of reduced potential of the highly sensitive stem cells, progenitor cells and proliferating-maturing cells, especially under late chronic exposure. It should be noted that the impairment of the hemopoietic function observed late after exposure can be associated not only with organic changes (e.g., bone marrow hypoplasia and vascular changes) but also with the limited capacity for physiological regeneration of the hemopoietic tissue due to depletion of the stem cell pool.

However, the results of our observations on the hemopoietic system status in residents of the Techa riverside villages indicate that the mechanisms of adaptation to the long-term chronic exposure in the range of low to intermediate doses are sufficiently effective. As shown by our study, the mechanism that the hemopoietic tissue tolerates chronic exposure is of intricate nature as it is associated with a complex of subcellular, cellular, tissue, system and organism reactions to chronic exposure.

The following conclusions can be drawn from the results of 5-decade follow-up of the population exposed in the Techa riverside villages:

1. In the early years of chronic exposure (1951-1953) of BM dose-rate reaching 0.29 Sv/yr reduction in leukocyte (neutrophil and lymphocyte) and thrombocyte counts was noted. The peripheral blood cell composition started to recover after dose-rate had decreased to 0.11 Sv/yr (1954-1956).
2. During the period of maximum chronic exposure, the normal erythrocyte count was maintained owing to compensatory mechanism, which involved increased rate of proliferation and maturation of erythrocaryocyte. Peripheral blood granulocytopenia observed during the period of maximum radiation exposure was determined by delay in differentiation of bone marrow granulocyte at myelocytic phase, as well as by a significant increase in the frequency of lethal damage and defective mitoses.
3. Normalization of peripheral blood count occurred within different time periods after the start of exposure. Characteristically, lymphocyte, thrombocyte and neutrophil counts were restored to normal 4-6, 7-10 and 12-17 years later, respectively.
4. The factors that modify the process of blood cell composition recovery after chronic exposure include gender, age at exposure and the degree of hemopoiesis inhibition during the period of maximum exposure. At comparable BM doses leukocyte count

was observed to normalize later in women than in men. In individuals exposed to similar BM doses, recovery of leukocyte count occurred earlier in those aged 20 years or less than in those older. Recovery period was noted to be prolonged in individuals with more pronounced initial hemopoiesis inhibition (leukocyte count $< 3.3\times10^{9}$/L).

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