SIGNS OF LATE RADIATION-INDUCED GENOMIC INSTABILITY IN PERSONS CHRONICALLY EXPOSED TO RADIATION.

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Abstract. The Techa River Cohort includes about 30,000 individuals exposed to protracted IR resulting from releases of radioactive waste into the Techa River during the initial years (1949-1956) of operation of the Mayak nuclear facility (weapon-grade plutonium production) in the Southern Urals region of Russia. The exposure was of a two-component type: external γ-(contributed by ^{137}Cs) and internal radiation (mostly due to ^{90}Sr). The aim of this study was to assess the spectrum of intracellular markers to evaluate the adverse long-term health effects in hematopoietic system at late time after onset of exposure. The study group comprised of 1,117 exposed individuals (the mean cumulative dose to red bone marrow was 1.13 Gy) and 484 unexposed individuals.

An increased rates of genetic disorders (CD3-CD4+ lymphocytes, mutations in the Tp53 gene, dicentrics, and micronucleus) and of DNA single- and double-stranded breaks frequency and repair ineffectiveness were detected among residents of the Techa riverside villages at late time after the onset of chronic radiation exposure. Cell Parameters of the cell's functional status parameters (apoptosis, and cell cycle control) had a peak at small doses (about 0.3 Gy), and a decline at higher doses. Trade-off between decline in frequency of apoptotic lymphocytes and increased fractions of Chk2+ lymphocytes for doses above 1Gy was observed. The oxidation status showed a sharp reduction in the concentration of Cu/ZnSOD (at doses below 0.3 Gy) and re-distribution of free active species in favor of their most aggressive forms. We interpreted these results as a manifestation of radiation induced genomic instability. Genomic instability has developed in a small proportion of subjects who should be assigned to the group at high risk of cancer development. The application of the mechanistic modeling will allow individualization of the radiogenic risk of leukemia based on the results of the study.

KEYWORDS *cellular effects, chronic radiation exposure, late effects, genomic instability, intracellular barrier mechanisms*

Introduction

The significance of the research on the effects of chronic exposure in man, especially in the light of the recent accident at the Fukushima-1 APS, does not raise any doubts. Provision of medical assistance to the population, prognostication and prevention of certain late radiation exposure effects in such situations should be based on adequate knowledge of the processes going on in the exposed organism, its tissues and cells. Currently, these issues are mostly investigated in the framework of epidemiological or experimental studies [BEIR VII Phase 2. 2006, Goans et al. 2001, Goodhead et al. 2009, Jeggo 2009, Morgan et al. 2009, Seed et al. 2002]. Clinical-laboratory studies on the late effects of radiation exposure in a heterogeneous population are not numerous. Basically, these studies focus on cohorts of apparently healthy workers of the nuclear-energy facilities due to easy accessibility of the data. However, because of the healthy worker effect, these data should be used with caution for medical prognostication in case of exposures of large populations heterogeneous over age, gender and health status.

Individual properties of the organism can play a key role in the development of late health effects resulting from chronic exposure. Since radiation-induced effects develop within a considerable time interval, physiological changes in the organism, involutional processes, in particular, can play an

essential role. At late time after the onset of exposure, the contribution of non-target reactions, primarily that of radiation-induced genome instability, may become a determinative factor [Limoli et al. 2000, Little 2003, Huang et al. 2003].

A unique opportunity to obtain information on the mechanisms involved in the development of chronic radiation effects are offered by the studies of the chronically exposed population of the Techa riverside villages (Southern Urals, Russia). The Techa riverside area is the only region in the whole world where beginning in 1949 a significant number of population were exposed to radiation in a wide range of doses for a long time period as a result of operations of the Mayak combine [Akleyev and Kisselyov 2002]. Maximum cumulative doses to red bone marrow (RBM) in individual members of the exposed population reached 9 Gy, and were accumulated in principle in the first years of exposure [Degteva et al. 2009, Tolstykh et al. 2011].

The exposure was of a combined nature and consisted of two components: external gamma- and internal, primarily due to ^{90}Sr. Dose rates of external exposure decreased exponentially over the first 6 years after the start of exposure. Dose rates to RBM due to ^{90}Sr decreased much more slowly which is associated with the peculiarities of ^{90}Sr metabolism in the human body [Akleyev and Kisselyov 2002]. The annual dose rate of radiation exposure received by the population did not exceeded the background levels over the recent decades.

The results of epidemiological studies are indicative of an increased risk of radiation-related malignant tumors, especially leukemia [Akleyev and Kisselyov 2002]. The hemopoietic system, due to its high radiosensitivity and the bone-seeking properties of ^{90}Sr, is a critical system in the case of radiation exposure on the Techa River. Based on our previous studies [Veremeyeva et al. 2010], we came to the conclusion that a number of changes observed in the peripheral blood cells of exposed persons are indicative of certain abnormalities at the molecular and sub-cellular level. The purpose of the present study is to investigate in more detail the radiobiological effects manifested by the hemopoietic system in persons exposed on the Techa River.

Materials and Methods

Fasting blood samples were taken at the clinical department of the Urals Research Center for Radiation Medicine in sterile conditions. Samples were labeled using sequence numbers from 1 to *n*, where *n* is the number of patients whose blood samples were taken on a specified day. A patient could only be identified by the physician who administered the assay while laboratory technicians had no access to the patient's individual information.

Standard research methods were used:

Analyses of unstable chromosome aberrations (dicentrics). The analysis of unstable chromosome aberrations was carried out on conventional painted slides. Peripheral blood lymphocytes were cultivated for 52 hours with 4,5 ml RPMI 1640 supplemented by 15% fetal calf serum, 2,5% PHA at 37^0C (Paneco, Moscow). Colchicine (0.1 μg/ml) was added before the final 3 hours. Chromosome preparation was performed according to the standard procedures [IAEA, 2001].

Assessment of the frequency of somatic mutations in lymphocytes (CD3-CD4+ cells). The method is based on reactions of CD3 and CD4 receptors on the surface of T-lymphocytes using the corresponding fluorescence-labeled monoclonal antibodies [Kyoizumi et al., 1990]. Blood was drawn using syringes containing heparin. Peripheral blood lymphocytes were isolated in sterile conditions on density gradient of Polysucrose 400 (at a density of 1.077-1.080 g/mL). Isolated cells were washed three times in Hanks solution at room temperature. Mononuclear antibodies IgG1/IgG1(BD Biosciences Pharmingen) (10 μl) were added into one of the test tubes, and 10 μl of mononuclear antibodies CD3/CD4 (BD Biosciences Pharmingen) were added to the other one. The use of mon/abs IgG1/IgG1 in combination with mon/abs CD3/CD4 was necessary for identification of the CD3-CD4- zone. The test tubes were vortexed for 3-5 sec., then they were kept in a dark place for 15 min. After incubation, 300-400 μl of PBS were added. Cells were scored using a laser (argon laser 488 nm) flow cytofluorometer (EpicsXL-MCL, Beckman Coulter).

Assessment of the frequency of DNA single- and double-strand breaks. Lymphocytes were isolated from venous blood on ficoll-urografin density gradient, washed twice in PBS and re-suspended in the culture medium. The suspension was irradiated at a dose of 10 Gy and dose rate of 0.7 Gy/min. Before

irradiation, immediately after it, and 2 hours after irradiation, the cell suspension was fixed in low-melting agarosa on a glass slide and kept in a lysing solution (2,5 M NaCl; 100 мМ EDTA; 10 мМ Tris; 1% Triton X-100; 10 % DMSO; pH 10) for 1 hour at +4°C. A horizontal electrophoresis was performed in constant electric field under alkaline [Singh et al. 1988.] or neutral conditions [Olive et al. 1990, Ostling et al. 1984, Singh et al. 1988, Miklos et al. 2009]. Finished preparations were stained using acridine orange (15 mg/ml) and visualized under a fluorescence microscope Axio with excitation filters 490-505 and 560-580 nm, and a blocking filter 515-545 and 600-650 nm. Om each glass slide at least 50 cells were scored, photographed and analyzed using vision systems based on a digital camera "Comey Imager". The tail density (% of DNA in the tail) was taken as the key parameter of DNA fragmentation. The alkaline version of the protocol was used for estimation of the number of single-strand and double-strand DNA breaks, and the neutral one – only for double-strand breaks. In order to assess the effectiveness of repair of DNA breaks, the tail density was assessed 2 hours after exposure as percentage of density registered immediately after in vitro irradiation. Accordingly, CIRD is the criterion of inefficient repair of DNA double-strand breaks, and CIRS is criterion of ineffective repair of DNA single-strand breaks.

Micro-nuclear test. The method is based on the following phenomenon: in the presence of a damaged chromosome at the stage of anaphase, chromatides advance to the opposite poles; acentric fragments of chromatids, chromosomes and whole chromosomes may not take part in the restoration of the nucleus at the telophase stage. They are inserted in daughter-nuclei and form secondary nuclei of smaller size which remain in the cytoplasm along with the main nucleus (Countryman and Heddle 1976). 0.7 ml of heparinized (15 u/ml) blood, to which 3.3 ml of RPMI medium with a 20% bovine serum, 100 μg/ml of steptomycin, 100 μg/ml of penicillin, 7 μg/ml of PHA were added, was incubated in a thermostat at 37^0C. Forty-eight hours after start of cell stimulation, cytocholasin B concentrated to 6 μg/ml was introduced, and the culturing then continued for 24 hrs more. After the termination of culturing, cells were treated with a cold hypotonic solution 0,125 M KCl, fixed in a mixture (3:1) of methyl alcohol and acetic acid, and spread on glass slides. Dried smears were painted after Giemza. Cells with micronuclei were identified among bi-nuclear cells (‰) and scored.

Evaluation of oxidative processes (contents of nitrogen and its metabolites, malonic dialdehyde), *status of the antioxidant system* (content of Cu/Zn-superoxide dismutase). The enzyme Cu/Zn SOD was determined using the method of immunoenzymatic assay (IEA) on Bender MedSystems plates (Austria). Concentration of enzyme Cu/Zn SOD in the blood serum was measured using the immunoenzyme assay. Cu/Zn SOD was bound by antibodies immobilized in microplate microwells Bender MedSystems. A conjugate of monoclonal antibodies to Cu/Zn SOD with horseradish peroxidase added to the microwells bound the complex Cu/Zn SOD-antibody. After the microwells were washed of unbound conjugate, a TMB substrate solution was added which interacted with the enzyme complex and formed a colored solution. The intensity of coloring was measured on the microplate photometer Multiskan Plus Plate Reader for 96-well microplates (Labsystems, Finland) at a wave length of 450 nm. Concentration of the enzyme Cu/Zn SOD in the samples studied was determined by extrapolation involving construction of a calibration graph based on 7 standard Cu/Zn SOD dilutions.

The levels of nitric oxide (NO) in deproteinized blood serum were measured using the test-system R&D Systems (USA) (Miles, 1996). NO_2^- concentration was measured using a 2-step Griess Reaction following transformation of NO^{3-} into NO^{2-}. Total NO concentration was calculated as a sum of concentrations of NO-metabolites. Measurements of color intensity and calculations of the test results were performed using Elise Analizer «Multiskan Plus» (Labsystems, Finland, wave length 540nm).

The intensity of membrane lipid peroxidation (LPO) was assessed based on the secondary product of LPO, malonic dialdehyde (MDA), reacting to thiobarbituric acid [Kamyshnikov 2000].

TUNEL method: assessment of the frequency of lymphocyte apoptosis. The method is based on interaction of fluorescence-labeled oligonucleotide sequences with 3'OH areas of DNA fragments [Enari et al., 1998]. To estimate the frequency of apoptosis, the cell suspension was divided into three parts and placed in 3 test tubes. Studies of apoptosis frequency were performed at different time intervals after samples of venous blood were collected: baseline level, 5 hours later, and 24 hours later. Incubation was performed in a standard culture medium at 37°C (4ml PRMI-1640, 5% fetal calf

serum, PHA 0,5%) [Rowland-Jones and McMichael, 2000]. The cell suspension was permeabilized using 4% paraformaldehyde, fixed using 70% alcohol, and painted according to the protocol Apo-Direct KIT BD Pharmingen Technical Data Sheet метода TUNEL [www.bdbeurope.com/temp/usextfile5182.pdf]. Scoring was done using a laser (argon 488 nm) flow cytofluorometer (EpicsXL-MC) with a wave length of PI 618 nm, and FITC 502 nm.

*Identification of specific features of the lymphocyte cell cycle (immunofluorescent painting of the intracellular proteins Chk2 and Ki-67). S*pontaneous level of lymphocytes containing protein Chk2 [Matsuoka et al.,1998; Tominaga et al., 1999]. Peripheral blood lymphocytes [Prussin et al. 1995] were fixed (Cytofix/Cytoperm and Perm/Wash Buffer; BD Biosciences) and painted using primary (anti-Chk2 (human) BD Biosciences) and secondary FITC-labeled (FITC IgG1 (anti-human); BD Biosciences). To assess spontaneous levels of proliferating was performed fixation and permeabilization (Cytofix/Cytoperm and Perm/Wash Buffer; BD Biosciences) and stain anti-Ki-67 human kit ; BD Biosciences) and PI\RNasa (Kubbutat, 1994). Scoring was done using a flow cytofluorometer EPICS MCL- XL (argon laser 488 nm, 15 mW). Number of cells scored: no less than 10 000.

Reproducibility of the method was tested by conducting 2 replications of samples selected using a randomized approach.

Statistical methods of analysis. The following variables were considered: CD3-CD4+ lymphocytes, dicentrics, Micronucleus, Mutations in Tp53, DNA double-stranded breaks, DNA single-stranded breaks, Double-stranded break IRC, Single-stranded break IRC, Cu/Zn SOD, Nitrate (NO_3^-), Nitrite (NO_2^-), apoptosis, *Chk2 and Ki-67)* For each variable the means, standard errors, and standard deviations were calculated in each of five dose groups: i) 0 mGy (control), ii) 0-299 mGy, iii) 300-599 mGy, iv) 600-999 mGy, and v) 1000+ mGy.

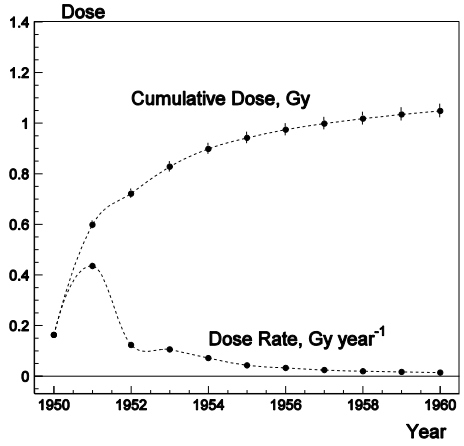

Figure 1. *Dynamics of dose rate and cumulative dose (RBM)*

Characterization of the study population

The study group comprised 1117 individuals chronically exposed to radiation due to production activities at the *Mayak NF, (The Southern Urals, Russia*) and 484 unexposed individuals (controls). The groups were comparable in terms of gender, age, and ethnicity, e.g., mean age for exposed individuals was 67.3 (SE=0.22) years and 67.1 (SE=0.30) years for unexposed control. Red bone marrow (RBM) exposure doses were estimated using the dosimetry system TRDS-2009 [Degteva et al. 2009, Tolstykh et al. 2011]. The dynamics of dose rates and dose accumulation for the study group members is shown in Fig. 1.

Results

The empirical analysis of the relationship between the changes observed and the dose to RBM has allowed detection of dependences shown in Figures 2 and 3. Analysis of distributions for investigated parameters allowed for conclusion that the differences between the exposed and unexposed subjects in terms of mean values of the parameters reflecting a damage to genome are by no means always determined by their high values in all dose groups. If the frequency of mutations in Tp53 is significantly higher than that for the controls at minimum doses (up to 0.3 Gy), the frequency of somatic mutations (mutations in TCR genes) is monotonously increasing in all dose groups, however, statistical significance is only reached in the dose group 0.6 Gy – 1 Gy. The lack of differences revealed by a comparison of all groups of exposed and unexposed individuals does not always reflect the true facts either. In spite of the absence of significant differences shown through comparisons of mean values of frequency of dicentrics and micronuclei, we can see that the dose

dependence does exist: in the dose group > 1 Gy the level of both micronuclei and dicentrics is sufficiently higher, and the differences are close to statistical significance. At doses below 0.3 Gy, a tendency to a decrease in the proportion of cells with micronuclei was observed as compared to that demonstrated by the unexposed controls.

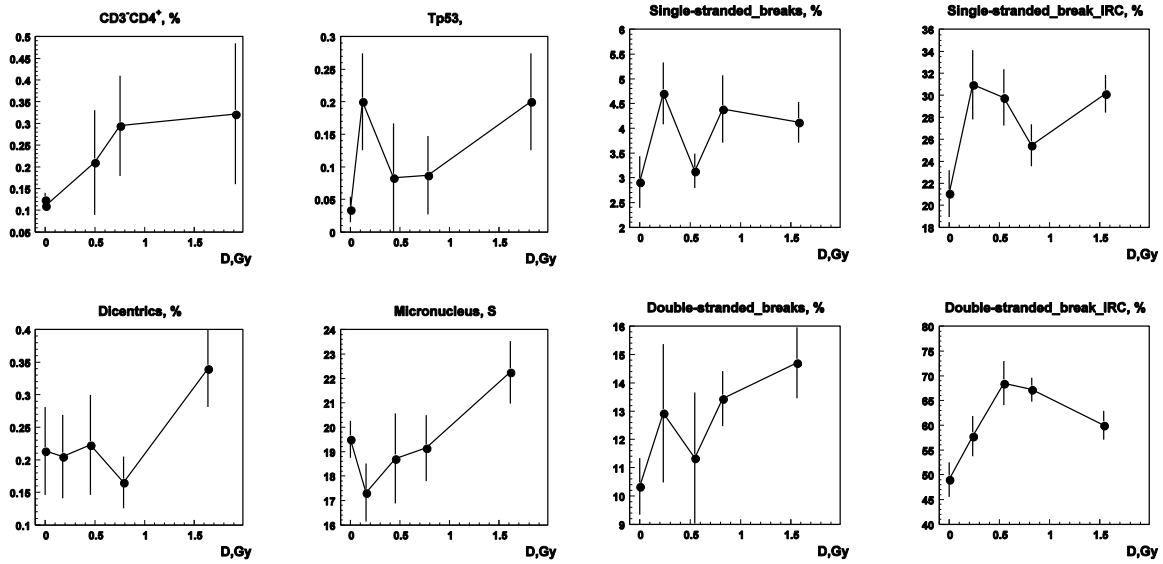

Figure 2. *Dose dependence of frequencies of genetic pathologies*

Obviously, the cause of any mutation/aberration is the disruption of DNA continuity. In our study we estimated the frequency of single- and double-strand DNA breaks. The number of single-strand breaks (percentage of migrant DNA) already increased at doses of up to 0.3 Gy. A similar graph was obtained for estimation of the number of mutations (in Tp53 gene, somatic mutations in TCR). The same situation was observed when the number of DNA double-strand breaks was assessed, however, the statistical significance of the differences was only noted at doses above 0.6 Gy, though the increased frequency of breaks, as can be seen from the plot, is observed even at doses up to 0.3 Gy. On the whole, this graph is similar to the graphs describing the dose dependences of the frequency of chromosome aberrations and micronuclei.

However, the number of mutations\aberrations is determined not only by the number of DNA breaks, but also by the effectiveness of repair. It can be seen from Fig. 1 that after a standard ex vivo irradiation at a dose of 10 Gy and a subsequent incubation for 20 hours the proportion of unrepaired DNA in peripheral blood lymphocytes is essentially larger even in the minimum-dose group in terms of repair of both single- and double-breaks. For most dose groups the values differ significantly from those for the controls, or the differences are manifested as a tendency.

When dose rate values are for a long time retained at levels corresponding to the natural background, oxidative stress and ineffective functioning of the antioxidant system can be considered as a most probable cause for an increased number of DNA breaks in exposed individuals. As is shown in Fig. 3, even in the dose group below 0.3 Gy a significant decrease is observed in Cu/Zu SOD concentrations, one of the key enzymes of the antioxidant system. At doses above 0.6 Gy, Cu/Zn SOD concentrations reach the levels registered in the controls.

We estimated the oxidative status based on the concentrations of malonic aldehyde as well as active forms of nitrogen: nitrate and nitrite. The graphs describing the dose characteristics of the malonic dialdehyde and nitrate concentrations are quite similar. A decline of concentrations of NO3-, even at minimum doses, and a slight increase in malonic dialdehyde at doses 0.3-0.6 Gy and at 0.6-1.0 Gy, followed by a decrease compared to that in the controls were observed. The changes in nitrite concentrations are occurring in a quite different way. It increases dramatically at doses up to 0.6 Gy,

persists at about the same level and and increases insignificantly at doses above 1 Gy. There is no doubt that a study of a wider spectrum of antioxidants and radicals is needed, however, the values we have chosen demonstrate that there occurs a non-linear dose-dependent decrease in antioxidant concentrations and a re-distribution of free radicals with a shift to increased concentrations of their

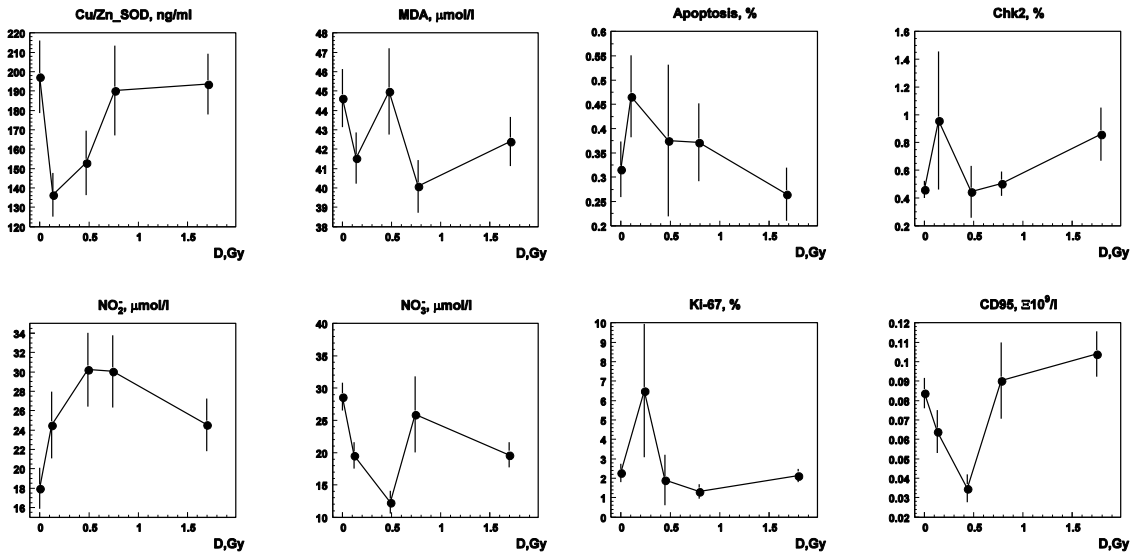

Figure 3. *Dose dependence of states of antioxidant and functional systems*

most aggressive forms.

Thus, it can be concluded that exposed individuals show signs of oxidative stress associated with inadequate functioning of the antioxidant system and the occurrence of single and double-strand DNA breaks characterized by a less efficient repair as compared to that seen in unexposed subjects. It should be noted that the changes are building up even at small doses, and later on they persist at the same level, as a rule.

In our opinion, it would be important to investigate the functional intracellular mechanisms of resistance to the processes of accumulation of mutation in the cell, transfer of genetic alterations to the progeny and the advance to malignization which are observed in cases of increased numbers of DNA breaks and reduced effectiveness of repair processes, and also the exposure dose dependence of these mechanisms.

As was indicated above, cell cycle delays for lymphocytes were evaluated based on the proportion of Chk-2-positive cells in the blood. An increased number of these cells was found at doses above 1 Gy. An increase, even if insignificant, was noted for the dose subgroup up to 0.3 Gy.

Apoptosis which is one of the key mechanisms of damaged cell elimination was studied in more detail. In spite of the lack of significant differences between exposed and unexposed subjects at doses below 0.3 Gy, an increase in the proportion of apoptotic peripheral blood lymphocytes (which does not reach statistical significance) is observed which is followed by a decrease down to the values seen in the controls, and even lower.

The graph showing the dose dependence of apoptosis is very similar to the graph of the analogous dependence for frequency of Ki-67 positive lymphocytes. At doses no more than 0.3 Gy an increase in this value is observed. A decrease in the value, interpreted as a tendency at doses above 0.6 Gy, is noted among members of the dose groups more than 0.6 Gy. Presumably, a compensatory proliferation of lymphocytes on the periphery or/and lymphocytes at early phase of apoptosis are identified. Thus, the research into the functional processes going on in the cells shows a similar bell-shaped type of dose dependence. At its lower values, an activation of the protection mechanisms is observed for all parameters studied, while at increased doses of 1 Gy or higher, a certain trade-off between a decrease in the proportion of eliminated lymphocytes and accumulation of lymphocytes in gross arrest can be noted.

Discussion

Thus, it can be inferred that decades after the dose rates had decreased to the background level, changes are still retained in the peripheral blood of exposed people. Chronically exposed persons manifest an increased frequency of mutations, aberrations of unstable type and micronuclei. However, if the level of mutations in exones 4,5,7 and 8 of Tp53 is increased even at minimum dose values (up to 0.3 Gy), the frequency of phenotypically manifested mutations (mutations in the TCR) is increased in the dose group of above 0.6 Gy. The frequency of gross damage to genome (aberrations and micronuclei) does not actually change at doses below 1 Gy. The frequency of micronuclei in the low dose subgroup was even found to be decreased as compared to that in the controls. The critical point is a dose value close to 1 Gy at which an essential increase in chromosomal and radiation-induced aberrations is observed. Thus, the worse the genetic damage, the higher the dose at which an increased frequency of damage is manifested. The causes for this phenomenon may be either a higher stability of the double-strand brake repair system or a quick elimination surpassing accumulation of the cells with damage.

Evidently, the damage of the DNA molecule, its single- and double breaks, in particular, are underlying the changes registered in the genome. Even at doses up to 0.3 Gy we noted an increased number of single-strand breaks. The graphics of dose dependences of single-strand // mutation frequency (Tp53, TCR) are similar in shape. The increase in the number of double-strand beaks is almost linear in shape reaching maximum values at doses above 1 Gy. The assumption that chromosome aberrations are caused by double-strand DNA breaks correlates with the evidence about accumulation of aberrant cells specifically in this dose group. The effectiveness of repair of both types of DNA breaks is significantly reduced even at doses below 0.3. Besides, free-radical processes may lead to disruption of the DNA molecule continuity [Ogawa et al. 2003].. In our feasibility study we evaluated the status of the antioxidant system and occurrence of oxidative stress in exposed subjects. Indeed, even at doses below 0.3 Gy it was noted that the SOD concentration had decreased more than 1.5 times which was indicative of insufficiency of the antioxidant system. The concentration of malonic dialdehyde as well as nitrate-anion more that 3-fold was registered. Both the former and the latter are more stable than nitrite-anions; the concentration of nitrite-anions is building up even at doses below 0.3 Gy, and it increases almost 2-fold in the dose group 0.3-0.6 compared to the values shown by the controls. We regard these changes, viz., functional disturbances of the antioxidant system and redistribution of free radicals in favor of more aggressive forms [Chen et al. 2001, Limoli et al. 2003, Ogawa et al. 2003] as evidence of oxidative stress.

The characteristics of the status of intracellular mechanisms protecting the organism from accumulation of cells with genetic damage can also demonstrate dose-dependent changes. The dose dependence has a bell-shaped form. The group with doses below 0.3 Gy was noted to manifest a certain activation of apoptosis of peripheral blood lymphocytes, increased proportion of lymphocytes with a cell cycle delay and the number of proliferating lymphocytes. At higher doses the number of proliferating does not differ from that observed for controls. In the dose group >1 Gy a trade-off can take place between a decrease in the frequency of apoptotic cells and increase in the proportion of cells with a delayed cell cycle. It can be suggested that the probable causes for this phenomenon этого are genomic instabilities, both radiation-induced and occurring subsequently, such as genetic, epigenetic, and proteomic abnormalities in apoptotic pathways. Probably, it is a compensatory response of the system which prevents a build-up of deficit of cells eliminated through apoptosis and which switches the cell's reaction to the damage from the elimination pathway to the pathway of preservation in the state of gross-arrest. In case of small doses, when barrier actvation occurs, no essential increase in the frequency of mutations/aberrations is observed (except for mutations in Tp53); a certain decrease in the frequency of micronuclei may even be seen. At high doses, on the contrary, inhibition of apoptosis, accumulation of apparently defective cells in thr stae of gross-arrest and the highest values of pathplogical characteristics have been observed.

Obviously, the accumulation of cells with genetic damage and in the state of cell gross arrest cannot be ignored by the organism, and it can be regarded both as acceleration of involutional processes and as a cause of increased risk of cancer development.

A combination of the changes observed at the biochemical and macromolecular level and increased levels of mutations/aberrations has shown that members of the Techa River population

manifest signs of radiation-induced genomic instability even now, about 6 decades after the onset of chronic radiation exposure of the population. It can be assumed, taking into account the fact that increased frequency of chromosome aberrations was registered as a result of earlier studies as well [Petrushova et al., 1993], that the genome instability has been developing over the whole period of the follow-up of the Techa River Cohort, and it may be one of the major causes for increased radiogenic risk of malignant neoplasms, including leukemia, [Krestinina et al., 2005]. Genetic abnormalities seen in progenitor cells of the red bone marrow and blood lymphocytes could cause breakdowns in functional processes in cells that did not lead to lethality or obstruct further proliferation of cells. At the same time, the sensitivity of the progeny of these cells to external factors increased probabilistically. Thus the mechanism of genomic instability was set in motion.

Under the conditions involving some genetic abnormalities already existing, any stressful effect on the cell may lead to further advancement of the cell towards malignization. During the period of the studies on an aging cohort, involution processes which can exert both additive and synergic effect play an important role. Based on the above-stated, we take the liberty of suggesting that under chronic exposure in man two scenarios of leukemia development are possible: an early increase of risk associated directly with exposure at relatively high dose rates which decrease after cessation of exposure, and a late increase of risk during the period of increased age-related changes associated with advanced genomic instability.

A question may be raised: why haven't people with increased levels ogf mutations and aberrations, with functional insufficiently of intracellular protective mechanisms developed cancer yet? It is hardly possible to provide an unequivocal answer to this question. Firstly, mutation/aberration is a probabilistic process, and mutations pushing the cell to malignization occur very rarely. Secondly, apart from intracellular processes we have investigated, there are innumerable other mechanisms for maintaining the genetic homeostasis in the organism, including the immune system. In our previous study we received evidence which is indicative of activation of the immune system , but the data are not included in this paper. At doses over 1 Gy **,** the proportion of CD95+ cells increases, presumably, as a compensation for p53-mediated inconsistency of apopotosis. This fact in combination with a substantial increase in TNF concentration (a significant increase is seen already at doses below 0.3 Gy) as well as a number of cytokines IFγ, IL1, IL2, IL6 in the blood of chronically exposed persons provide evidence of such activation

A peculiarity that cannot be omitted in this work is almost complete identity of graphs describing dose dependence of mean values for sub-groups, and graphs describing the proportion of individuals with excess values relative to the referent values. It can be inferred from the foregoing that the mean values are determined not by the changes in the values for each group member, but by an increase or a decrease in the proportion of individuals with values exceeding the referent values. Actually, it is the matter of the presence in the exposed population of a group of individuals differing in terms of sensitivity to radiation exposure from most of the cohort members. It may be either a higher radiosensitivity, if the intensity of mutation processes increases, or a higher radioresistance is activation of protective mechanisms, such as apoptosis, and cell cycle control, are meant..

In the publications [Akushevich et al. 2012, Veremeyeva et al. 2009] we considered the possibilities for mechanistic modeling of carcinogenesis and individualization of cancer risk individualization. The concept of the model suggested is based on the assessment of the status of intracellular mechanisms for protection from damage to the genetic material and fixation of mutations/aberrations in the cell's progeny. The study provides additional material for further development of this research area.

Conclusions

Thus, at late time after the onset of chronic radiation exposure an increased rate of genetic disorders was noted among residents of the Techa riverside villages. The increased frequency of mutations/aberrations is, evidently, related to genomic instability which developed as a late response to radiation exposure. The phenomenon is associated with ineffective functioning of such intracellular mechanisms as antioxidant system, repair of DNA breaks, cell-cycle control and apoptosis.

The dose dependence of the changes observed is determined by an increase in the number of subjects with excess reference value rather than by deviations of individual values in most members of the dose sub-group. Evidently, genomic instability has developed in a small proportion of subjects who should be assigned to the group at high risk of cancer development.

The patterns of dose-dependence for mean values in the subgroups are determined by the type of the parameter studied: i) the values of parameters reflecting pathological processes increase with dose and reach the maximum at doses above 1.0 Gy, ii) the values of the parameters reflecting cell's functional status (except for the antioxidant system) at small doses (up to 0.3 Gy) are higher than the respective values obtained for unexposed persons; iii) trade-off between decline in frequency of apoptotic lymphocytes and increased fractions of cells in gross arrest (represented by Chk2+ lymphocytes) for doses above 1Gy was observed, and iv) the oxidation status is characterized by a sharp reduction in the concentration of Cu/ZnSOD, already at doses below 0.3 Gy, and by re-distribution of free active species for the benefit of their most aggressive forms.

Further progress in investigation of this problem can be achieved by analyses of observed patterns by the methods for mathematical modeling prove resulting in developing predictive models capable to individualization of radiogenic risk of malignant neoplasms (specifically leukemia).

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