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Bystander effects induced by serum from survivors of the Chernobyl accident

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Objective. To examine blood samples from survivors of the Chernobyl accident for evidence of persistent bystander factors or clastogenic factors and to look at the ability of melanin and melatonin, which are radioprotective agents capable of preventing bystander effects in cell culture to prevent toxic effects.

Materials and Methods. Serum was extracted from blood samples of control and test groups and added to human immortalized reporter cells, used in our laboratories for identification of bystander effects. These were then analyzed for evidence of micronucleus formation and viability.

Results. Micronuclei were significantly elevated in cells exposed to serum samples from Chernobyl liquidators and from workers in Gomel. Viability of cells treated with these sera was correspondingly reduced.

Conclusion. Twenty years after the accident at the Chernobyl Plant, there is still evidence of the presence of clastogenic or bystander factors in the serum of populations exposed to radiation from the reactor. © 2007 International Society for Experimental Hematology. Published by Elsevier Inc.

It is well accepted that cells, in response to radiation exposure, may release certain transmissible factors (i.e., bystander factors) capable of inducing cellular responses in unexposed cells. These factors have been reported to induce genomic instability and delayed death in cells that have not been exposed to radiation. They are also suspected of being involved in radiation leukemogenesis. For state-of-the-art reviews, see [1–4]. There is some debate as to whether bystander factors are related to “clastogenic factors.” The latter were first described in the plasma of persons who had been irradiated accidentally or therapeutically [5,6], and was also observed in atomic-bomb survivors, where they persisted for many years after irradiation [7].

Clastogenic factors are mixtures of pro-oxidants with chromosome-damaging properties and not single factors, as thought by the first observers [5,6,8,9]. Biochemical analysis identified peroxidation products of arachidonic acid, released from membrane phospholipids, cytokines such as tumor necrosis factor α , and unusual nucleotides, such as inosine di- and triphosphate. The clastogenic properties of these components of clastogenic factors were confirmed by cytogenetic studies of the corresponding commercial standards [10].

It has been reported that radiation exposure can result in the release of soluble (clastogenic) factors into the circulating blood that are capable of producing chromosome damage in cultured cells [6]. These factors may play a role in carcinogenesis [10]. They could be similar to the soluble factors described in the bystander effects measured using culture media transfer experiments [11]. There is evidence for radiation induction of these soluble factors, both in vitro and in vivo [12–14]. Bystander effects may have important biological consequences within an organism. Currently, they are thought to be part of a general stress response that is genetically controlled and mounts apoptotic or survival responses, depending on the radiation dose and the underlying genotype of the irradiated organisms or cells [13,15]. However, clastogenic effects are generally regarded as toxic and adverse long-term consequences of radiation exposure. It is obviously important to determine whether both effects are related. Antioxidants and other radioprotective agents, such as melanin and melatonin, have been shown to prevent bystander effects [16–18], although it is not clear whether they prevent factor production by the irradiated cells or response by the unirradiated recipients. For this reason, melanin and melatonin were added to recipient cells to address this question.

Recently, Emerit et al. [10,19,20] reported the presence of these factors in the plasma of workers and children exposed as a consequence of the Chernobyl reactor accident.

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Monitoring of these transmissible factors as overall indicators of biological response in human tissues and fluids could reflect levels of radiation-induced damage regardless of the specific targets being exposed.

In her research, Emerit [10] studied the influence of blood serum samples from Chernobyl liquidators on her patients' own frequency of lymphocyte aberrations. As this approach does not permit quantification of signal strength, because response and signal production are measured in the same genotype, we used a well-defined reporter cell line to measure signal strength in all samples. The human keratinocyte reporter cell line, immortalized by human papilloma virus (HPV), is a reporter system, which we treated with serum extracted from blood samples from different population groups (i.e., Chernobyl liquidators, people affected after the Chernobyl accident, Polesky State Radiation and Environment Reserve workers, liver cirrhosis patients, and acute virus-infected patients from contaminated territories of the Gomel region) and analyzed using cytogenetics, micronucleus assay, and Alamar Blue tests.

Materials and methods

Human population

The affected populations after the Chernobyl accident include three main categories: Chernobyl liquidators 1986–1987, workers from Polesky State Radiation and Environment Reserve (PSRER), workers and people living in territories of Gomel region contaminated by radionuclides. Doses to the liquidators would have been, on average, 15 cGy over a very short period. The chronic dose to the PSRER workers and residents of Gomel is impossible to estimate on an individual basis because of the variety of occupations and the number of isotopes released, but general information on deposition of these isotopes can be found in the United Nations Chernobyl Forum Report [21].

Blood serum extraction

The blood samples were taken and put into Vacutainers for serum extraction (BD, Franklin Lakes, NJ, USA), centrifuged at 2000g for 10 minutes, and the serum was frozen and stored at -20°C before use. Before freezing, the serum was filtered through Nalgene 0.22- μm filters in order to remove all residual cell components of the blood.

Cell culture of HPV-G cells

The HPV-G cell line is a human keratinocyte line, which has been immortalized by transfection with HPV, rendering the cells deficient in p53 [22]. They grow in culture to form a monolayer, display contact inhibition, and gap junction intracellular communication. Except where indicated, all reagents for cell culture were obtained from Gibco-Biotech (Irvine, Scotland).

HPV-G cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum, 1% penicillin-streptomycin, 1% L-glutamine, and 1 $\mu\text{g}/\text{mL}$ hydrocortisone. Cells were maintained in an incubator at 37°C , with 95% humidity and 5% carbon dioxide and were routinely subcultured every 8 to 10 days.

When 80% to 100% confluent, the medium was poured from the flask and replaced with 1:1 solution of versene (1 nM solution) to trypsin (0.25% in Hank's balanced salt solution; Gibco-Biotech) after washing with sterile phosphate-buffered saline (PBS). The flask was placed in the incubator at 37°C for about 11 minutes until the cells started to detach.

The flask was then shaken to ensure that all cells had been removed from the base of the flask. The cell suspension was added to an equal volume of RPMI-1640 medium to neutralize the trypsin. From this solution new flasks could be seeded at the required cell numbers.

Melanin

Melanin was isolated from animal hair by Belarus Pharmaceutical Association (Minsk). By analysis, it was determined to be eumelanin. Both orthochinoid and indolic fragments were present. Melanin was added to the cell medium at 10 mg/L concentration 1 hour before addition of test sera.

Micronucleus protocol

Micronuclei are structures containing chromosome fragments or whole chromosomes (sometimes groups of chromosomes) situated in the cytoplasm. At mitosis, they are not included in the nucleus because of the lack of a centromere (acentric fragments) or damage to fibers of the mitotic spindle or centromere (whole centromere).

After irradiation, micronuclei are formed in all cell types. The only condition of their formation is the passage of the cell through mitosis.

The main criteria for micronuclei detection include: micronuclei should have a similar structure to the nucleus; they should be smaller than the nucleus (less than one-half to one-third of the nucleus); they should be round and well-separated from the main nucleus; and the cell should have well-separated cytoplasm.

Blood serum from affected populations was added in a total volume of 5 mL culture medium to the flasks (24 cm²; 6000 cells per flask) 1 to 2 days after seeding, and cells were replaced in the incubators for 1 to 2 hours. Then cytochalasin B was added in 7 $\mu\text{g}/\text{mL}$ concentration, and cells were incubated for 24 hours. After this, the cell culture medium was removed, cells were washed with PBS and fixed with chilled Karnau solution (one part glacial acetic acid and three parts methanol, 10–15 mL three times for 10–20 minutes). Later, coverslips were dried and stained by 10% Giemsa solution. Using mounting medium (Sigma, Poole, Dorset, UK), coverslips were attached to the microscope slides.

The micronucleus count was carried out under inverted microscope ($\times 400$). Micronuclei were counted only in binucleated cells (1000 binucleated cells per flask)

Routine cytogenetic test

Lymphocytes cultured from the test groups were stimulated with phytohemagglutinin for 48 hours before assay. Three hours before the end of cultivation 30 μL colchicine was added. The cell culture was centrifuged for 5 minutes at 1500 rpm, supernatant was removed and cells were washed with warm 0.55% KCl solution (at 37°C) and incubated for 20 to 25 minutes. Cells were then fixed at -20°C using Karnau solution (one part of glacial acetic acid and three parts of methanol, 5 mL solution per flask). The fixative was changed three times after (10, 20, and 10 minutes).

Cells were mounted on microscope glasses (three to four drops of sample per glass) and dried at 40°C to 42°C. Cells were stained using 10% Giemsa solution for 8 minutes and after drying analyzed under light microscope. Only metaphases with a complete complement of chromosomes were analyzed.

Cell culture medium for microplate Alamar Blue assay

For the Alamar Blue assay, the cell culture medium should not contain phenol red sodium salt, because it is fluorescent and will interfere with the Alamar Blue dye. To prepare suitable medium, powdered Dulbecco's modified Eagle's medium nutrient mixture F-12 HAM (Sigma), 1.56 g was added to 100 mL deionized water (15°–20°C) and stirred until dissolved. To this solution was added 0.12 g sodium bicarbonate. After this, the pH of the medium was adjusted to 6.9 using 1 N HCl or 1 N NaOH. Then the medium was sterilized using 0.22- μ m filter and dispensed into a sterile container and stored at 2°C to 8°C in the dark. Before experiments, Alamar Blue dye was added to the medium to make a 5% solution.

Alamar Blue assay

Alamar Blue is a safe, nontoxic aqueous dye that is used to assess cell viability and cell proliferation. Using this assay, the innate metabolic activity of cells can be monitored. Alamar Blue is soluble, stable in culture medium, and nontoxic. Continuous monitoring of cells in culture is, therefore, permitted. Specifically, Alamar Blue does not alter the viability of cells cultured for various times, as monitored by Trypan Blue exclusion. Cells grown in the presence of Alamar Blue and subsequently analyzed by flow cytometry for CD44, CD45RB, CD4, and heat-stable antigen are found to produce similar numbers of viable cells and antigen-expressing cells as non-Alamar Blue exposed cells. The oxidized indigo blue, nonfluorescing form of this chromogenic indicator dye (Biosource, Camarillo, CA, USA) is reduced by cellular dehydrogenases, to a reduced pink fluorescent form. Proliferation measurements with Alamar Blue may be performed either spectrophotometrically by monitoring the absorption of Alamar Blue-supplemented cell culture media at two wavelengths using a standard spectrophotometer, or alternatively, proliferation measurements with Alamar Blue may be performed fluorometrically using a spectrofluorometer, or a microtiter well plate reader.

Cells were seeded in 96-well microplates (NUNC, Rochester, NY, USA) in quantity of 2×10^4 cells/well. After seeding, cells were incubated for 24 hours to attach to the bottom of the well. Then media was removed, cells rinsed with PBS and blood serum from affected after Chernobyl accident populations were added to the cells together with melanin and melatonin. Microplates were moved back to the incubators. Twenty-four hours later, serum was removed, cells rinsed with PBS and 100 μ L 5% (v/v) solution of Alamar Blue prepared in Dulbecco's modified Eagle's media was added. Microplates were moved back to the incubators. Three hours later, fluorescence as fluorescent units was quantified with a microplate reader (TECAN GENios, Grödigg, Austria) at the respective excitation and emission wavelength of 540 and 595 nm. Wells containing medium and acamar blue without cells were used as blanks. Mean fluorescent units for the three replicate cul-

tures for each exposure treatment were calculated and the mean blank value was subtracted from these results.

Results

Study of the effects of blood serum samples using micronucleus assay

Figure 1 and Table 1 present results obtained on the influence of blood serum samples from different population groups on micronuclei frequency of HPV-G cells. Control blood serum samples are taken from healthy people of the same age and gender. A second set of controls were HPV-G samples exposed to normal medium containing fetal calf serum instead of test serum. It can be seen that the frequency of micronuclei is significantly increased in the cell samples receiving serum from the liquidators and the workers at the contaminated Gomel site. There is also an elevated level in patients from the Gomel region with liver cirrhosis and particularly in the group with acute viral infections. The individual data are presented in Table 1 with the averages plotted on Figure 1.

Comparative analysis of cytogenetic aberrations and micronucleus frequency in populations affected after the Chernobyl accident

The influence of serum samples from victims of the Chernobyl accident (Chernobyl liquidators, PSRER workers, and residents of contaminated territories) on induction of genomic instability in human lymphocytes and the bystander effect in HPV-G cells was compared. The data showing the influence of serum samples on HPV-G cells (micronuclei test) and the cytogenetic aberrations in the blood lymphocytes of the same donors are shown in Table 2. It is clear that both endpoints are significantly higher in the exposed population compared with the controls. The individual data are plotted against each other in Figure 2 and

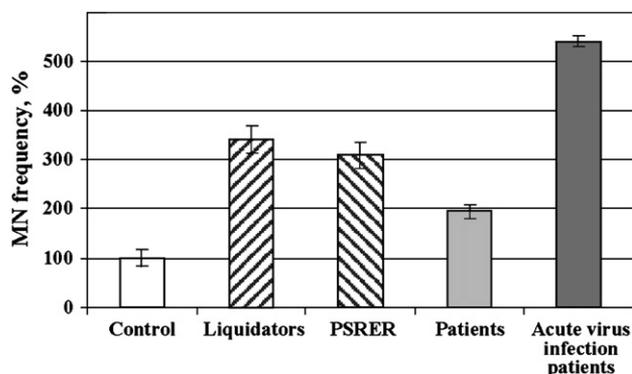


Figure 1. Cytotoxic effect of blood serum samples from healthy people, Chernobyl liquidators, and people affected after Chernobyl accident on HPV-G cells (control as nontreated cells, average data for all groups of populations. MN = micronuclei; PSRER = Polesky State Radiation and Environment Reserve (PSRER)).

Table 1. Individual measurements for the number of micronuclei in HPV-G reporter cells treated with serum from populations exposed to the Chernobyl accident and from controls

Individual micronuclei frequency (%)				
Group				
Control	Chernobyl liquidators	PSRER	Residents	Acute virus infection patients
31.01 ± 10.79	247.23 ± 17.17	301.45 ± 14.26	132.55 ± 10.59	326.43 ± 16.36
70.37 ± 15.57	313.95 ± 25.02	241.41 ± 13.41	73.34 ± 8.21	376.40 ± 16.24
71.69 ± 15.44	278.02 ± 17.09	209.89 ± 14.89	146.55 ± 10.95	574.37 ± 16.72
90.91 ± 14.25	315.79 ± 22.74	211.23 ± 14.92	183.95 ± 12.12	457.92 ± 16.47
67.92 ± 12.18	278.20 ± 15.86	304.99 ± 14.39	146.83 ± 10.96	—
103.57 ± 18.21	212.62 ± 12.75	292.99 ± 14.10	171.04 ± 11.74	—
66.42 ± 15.13	295.88 ± 13.97	271.97 ± 14.39	188.24 ± 12.24	—
55.35 ± 13.89	266.67 ± 28.54	227.06 ± 14.37	194.20 ± 12.30	—
112.71 ± 8.82	302.18 ± 25.63	300.00 ± 31.62	169.26 ± 11.70	—
107.82 ± 9.58	255.87 ± 21.14	343.48 ± 31.31	122.17 ± 10.28	—
105.47 ± 9.69	206.35 ± 22.80	285.00 ± 22.57	192.98 ± 12.32	—
—	339.42 ± 28.60	198.11 ± 17.31	—	—
—	243.24 ± 22.30	246.15 ± 26.72	—	—
—	353.91 ± 30.68	262.50 ± 28.40	—	—
—	299.12 ± 24.80	210.00 ± 23.52	—	—
—	295.65 ± 30.09	295.29 ± 22.72	—	—
—	228.57 ± 28.98	175.56 ± 17.93	—	—
—	223.08 ± 25.82	262.95 ± 27.79	—	—
—	291.19 ± 28.12	229.39 ± 25.17	—	—
—	278.88 ± 15.32	222.22 ± 26.67	—	—
—	262.47 ± 15.94	116.98 ± 19.74	—	—
—	232.56 ± 20.37	—	—	—
80.30 ± 13.14 ^a (mean)	273.67 ± 22.44 ^a	248.03 ± 20.77 ^a	156.47 ± 11.22 ^a	433.78 ± 16.45 ^a

PSRER = Polesky State Radiation and Environment Reserve.

^aAverage.

show a significant correlation between micronucleus formation in bystander reporter HPV-G cells and actual aberrations in the victim's blood.

Study of the effects of blood serum samples using Alamar Blue assay

Table 3 and Figures 3 and 4 present data obtained in study of the influence of serum blood samples from different groups of those affected after the Chernobyl accident and control healthy populations on viability of HPV-G keratinocytes using Alamar Blue assay. All populations groups were matched for age and gender ratio. The control is the viability of the HPV-G cells growing in normal culture medium with fetal calf serum instead of test serum. Data are presented as fluorescence units (FU). The individual data (Fig. 3) show decreased viability in cells receiving serum from the affected populations. The values are averaged in Figure 4. Table 3 confirms that the data are statistically different.

When viability of reporter HPV-G cells is plotted against the micronucleus endpoint (Fig. 5), it is clear that the correlation is significant and inverse. This implies that for individual subjects, high viability of cells is associated with low micronucleus frequency.

Effect of melanin

Melanin is a known radioprotector and was used here to see if it protected reporter cells against the effects of serum from the test groups. Results presented in Figure 6 suggest it did not reverse the bystander effect in these cells.

Discussion

The micronuclei frequency in the controls indicates that the level of spontaneous mutagenesis is comparatively low in HPV-G reporters. The data also show that the number of the cells with two and especially three micronuclei is very low compared with the number of cells with one micronucleus. People exposed to chronic radiation (PSRER) have an increased potential to produce clastogenic (bystander) factors in their serum, leading to a considerable increase in micronucleus frequency in the reporter cells (almost three times higher than the control level; 248.03% ± 20.77% compared with 80.30% ± 13.14%, $p < 0.01$). At the same time, an increase in the number of cells with more than one micronucleus was observed. Thus, data clearly indicate that chronic intensive radiation exposure significantly influences the level of clastogenic factor accumulation.

Table 2. Cytogenetic status of lymphocytes and micronuclei frequency in HPV-G cells treated with serum samples from the same patients (average data)

Parameters	Groups of populations		p Value
	Control	Victims of Chernobyl accident	
Results of cytogenetic analysis			
People analyzed	61	32	
Average age (y)	37.66 ± 1.5	41.74 ± 0.98	
Total no. of metaphases	11,179	8739	
Cytogenetic status (%)			
Single fragments	1.67 ± 0.12	5.78 ± 1.97	<0.05
Double fragments	0.81 ± 0.08	1.89 ± 0.1	<0.01
Dicentrics and rings	0.11 ± 0.03	0.31 ± 0.22	>0.05
Atypical chromosomes	0.02 ± 0.01	0.02 ± 0.01	>0.05
Polyploidic cells	0.08 ± 0.03	0.22 ± 0.19	>0.05
Aberrant cells	2.48 ± 0.14	7.59 ± 2.37	<0.05
Total no. of aberrations	2.63 ± 0.15	8.59 ± 2.94	<0.05
Results of micronucleus test			
Total no. of binucleated cells	5554	14,045	
Micronuclear frequency in cells (%)			
No. of cells with 1 micronucleus	69.50 ± 12.21	189.66 ± 20.74	<0.01
No. of cells with 2 micronucleus	5.00 ± 3.08	24.51 ± 8.05	<0.05
No. of cells with 3 micronucleus	0.26 ± 0.21	4.56 ± 3.14	>0.05
Total no. of cells with micronucleus	74.76 ± 12.69	218.69 ± 21.90	<0.01
Total frequency of micronucleus	80.29 ± 13.14	251.18 ± 22.96	<0.01

Similar results were observed after comparative analysis of the micronuclei frequency between the control group and the liquidators group (people exposed to acute radiation 20 years ago). Thus, the total micronuclei frequency of 273.7 ± 22.4 and frequency of cells with micronuclei of 235.6 ± 14.0 in cells treated with sera from liquidators blood samples is significantly higher than in the control group (in both cases $p > 0.01$). Also, an increase in the number of cells with more than one micronucleus was observed.

No significant difference was observed between results for liquidators and PSRER workers groups. This suggests that the effects of previous radiation exposure can be fixed 20 years after radiation exposure and is indistinguishable from the effects of chronic exposure to low doses. Thus, it is necessary to continue further thorough control and checkup of Chernobyl accident populations.

These results correlate well with the data from the Hirshima population [5]. The data on the bystander effect

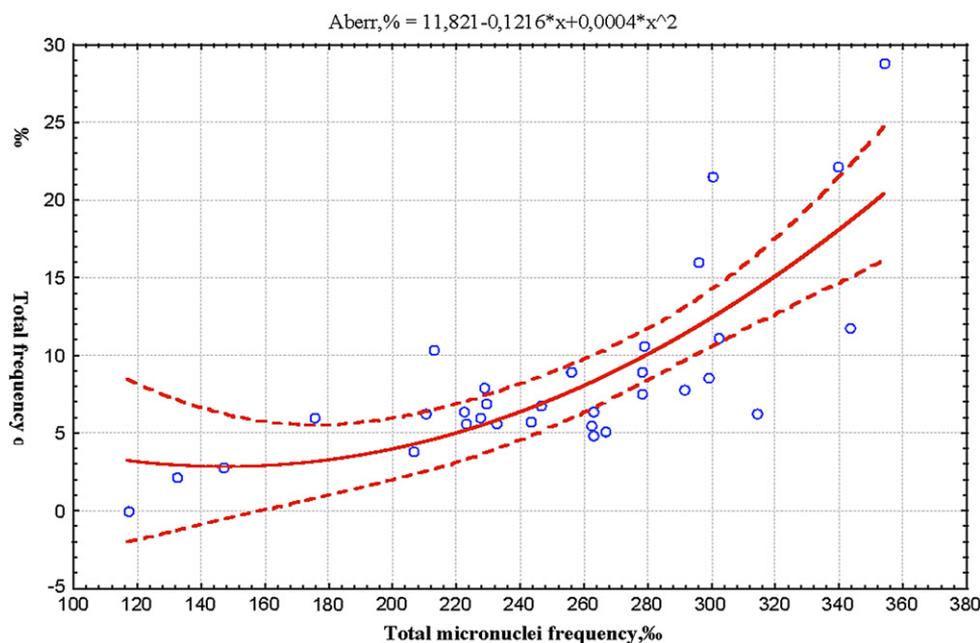


Figure 2. Correlation of micronuclei frequency with cytogenetic aberrations.

Table 3. Comparison of viability data from four independent groups (control, healthy people, liquidators, and residents of contaminated territories) using Mann-Whitney *U*-test

Groups of comparison	<i>U</i>	<i>Z</i>	<i>p</i> Value
Control vs Healthy People	21.00	0.39	>0.6
Control vs Liquidators	0.00	3.92*	<0.00001*
Control vs Residents	5.00	3.40*	<0.001*
Healthy People vs Liquidators	0.00	3.54*	<0.0005*
Healthy People vs Residents	4.00	3.07*	<0.005*
Liquidators vs Residents	35.00	3.03*	<0.005*

*Statistically significant at $p < 0.01$.

of the blood serum from PSRER workers confirms an earlier hypothesis [24] on the possible effects of chronic exposures to low doses of radiation.

The level of micronucleus induction cells receiving sera from the patients from high contaminated areas in Gomel with liver disease is statistically significantly different from control ($p < 0.01$), but much lower than in the groups exposed to acute radiation or the PSRER workers (in each case $p < 0.01$).

Interestingly, the level of micronuclei in cells treated with serum from the patients from the Gomel region with acute virus infection in active stage is higher than in all other groups. The micronucleus frequency induced by the serum from these patients is 435.6 ± 8.4 , and the number of the cells with micronuclei is 301.2 ± 7.8 . These figures are much higher than either the liquidators or the PSRER

workers. It is possibly the result of extremely high level of oxidants in active stage of acute virus infection.

These results show that the level of bystander activity of serum blood samples from people affected after Chernobyl accident may correspond to the level of activity of pathological processes, and that powerful oxidative stress from acute virus infection may substantially influence the level of bystander/clastogenic factors, thus creating a possibility of temporary destabilization of the genome of the somatic cells.

Summary analysis of micronucleus frequency in HPV-G cells after treatment with blood serum samples indicates that activity of serum samples from people affected after Chernobyl accident was almost three times higher than in control (251.18 ± 22.96 and 80.29 ± 13.14 , respectively; $p < 0.01$).

Experiments reported in this article also show that those affected after the Chernobyl accident have a significantly higher level of chromosome aberrations in their lymphocytes compared to controls ($p < 0.05$), as a result of a high level of nonspecific aberrations (single and double fragments, $p < 0.05$ – $p < 0.01$ compared to control, respectively).

At the same time, frequency of specific marker aberrations (dicentric and ring chromosomes) is almost three times higher compared to control, but not statistically significant. These data confirm data from Sevan'kaev et al. [24], showing that, with time, a significant part of these nonstable marker aberrations is lost, decreasing the effectiveness of biological evaluation of radiation doses.

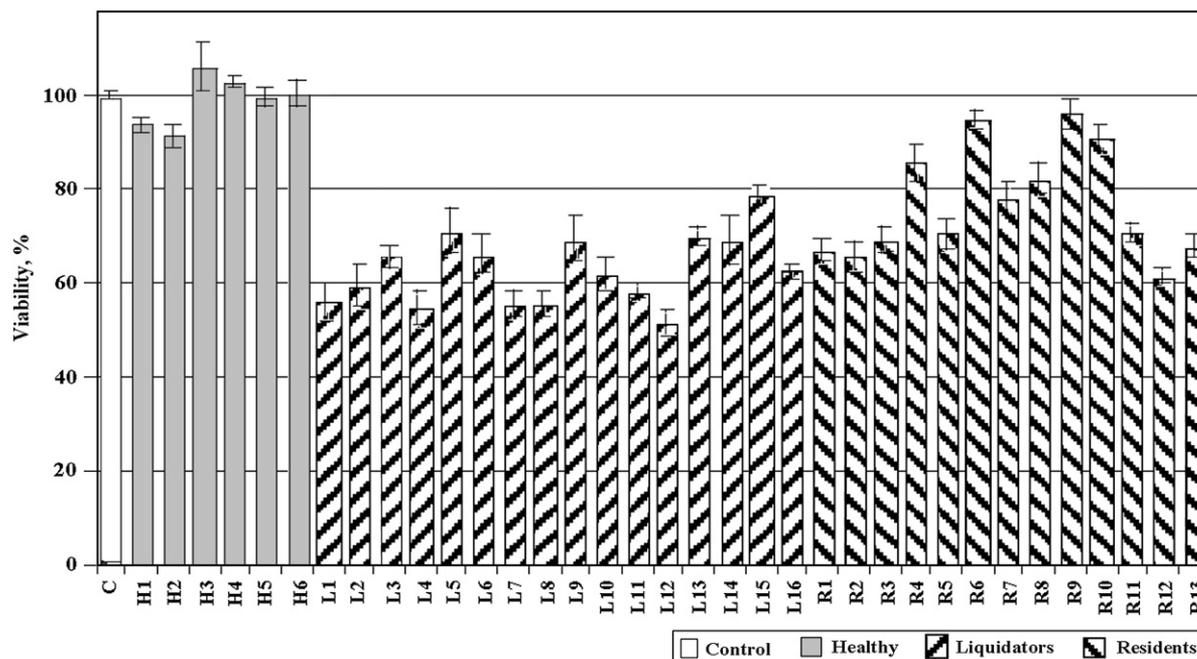


Figure 3. Cytotoxic effect of serum samples from healthy people, Chernobyl liquidators, and people affected after the Chernobyl accident on HPV-G cells (individual data for all groups of populations: control (C, nontreated cells), healthy people (H1–H6), liquidators (L1–L16), and residents from contaminated areas (R1–R13)).

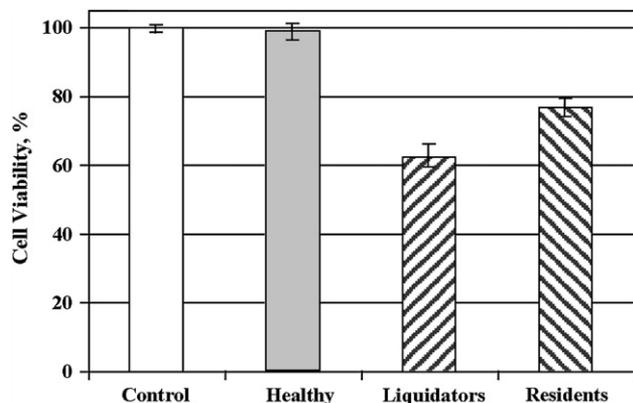


Figure 4. Cytotoxic effect of blood serum samples from healthy people, Chernobyl liquidators, and people affected after Chernobyl accident on HPV-G cells (control is nontreated HPV-G cells treated with normal medium containing fetal calf serum).

Thus, preliminary analysis shows that blood serum samples from people, even 20 years after the Chernobyl accident, may be reviewed as showing prolonged effects of radiation.

In order to understand interactions between parameters (total number of cells with aberrations and micronuclei and total frequency of aberrations and micronuclei), the dependence between them using polynomial analysis was studied [23].

From the data presented there is evident correlation between parameters. The strongest dependence is observed between total micronuclei frequency and total frequency of aberrations. Thus, the data analyses suggest that the level of damaging bystander factors in blood serum samples from people affected after the Chernobyl accident (micronuclei frequency) is closely correlated with the level of genome damage (aberrations frequency) in their blood lymphocytes.

As a consequence, our results allow us to suggest that total level of genomic instability may be due to both the level of radiation influence and individual radiosensitivity of people examined.

Thus, we conclude that the most affected group is liquidators of the consequences of the Chernobyl accident working at the site between 1986 and 1987 and exposed to acute irradiation. This group had, overall, the most expressed genomic instability and sharp increase in micronuclei and aberrations of different types. Less affected are PSRER workers, who continue to stay on in contaminated territories and are exposed to chronic radiation influence. Smaller effects are observed in residents of contaminated areas of Gomel region (dose-load is minimal), while the acute virus-infected patients represent a highly affected subgroup of residents.

A very interesting question in this field is whether, in some situations, cells affected by bystander factors die and are thus removed from the population. Mothersill et al. [13] showed that in mice irradiated in vivo, a clear

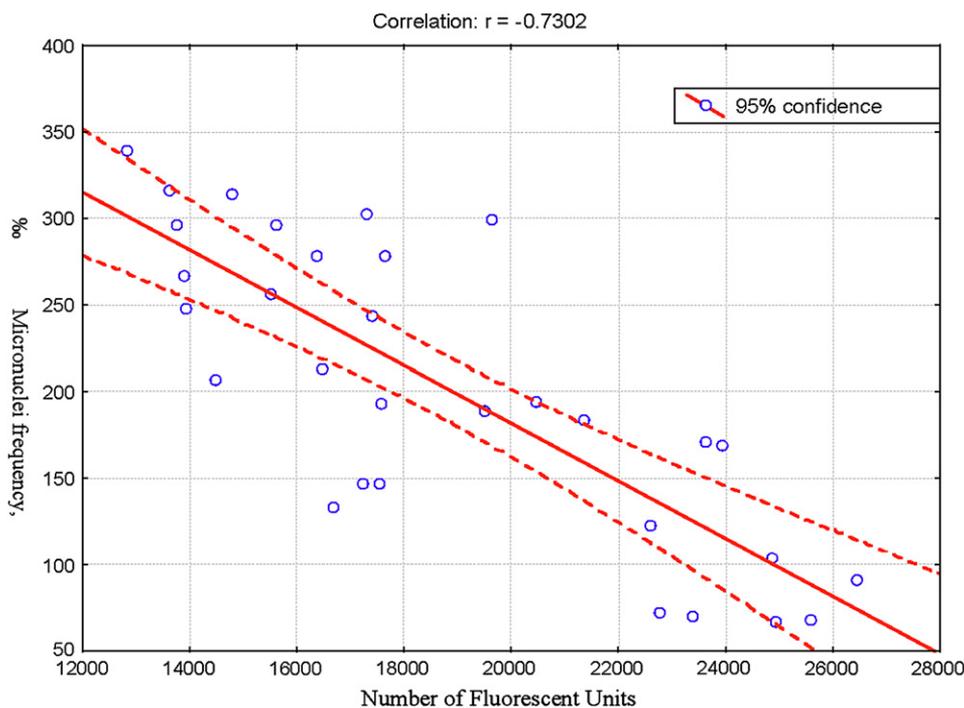


Figure 5. Multiple linear regression analysis of bivariate correlation between parameters micronuclei frequency and viability in human keratinocytes after treatment with serum samples from people affected after Chernobyl accident (r = coefficient of Pearson).

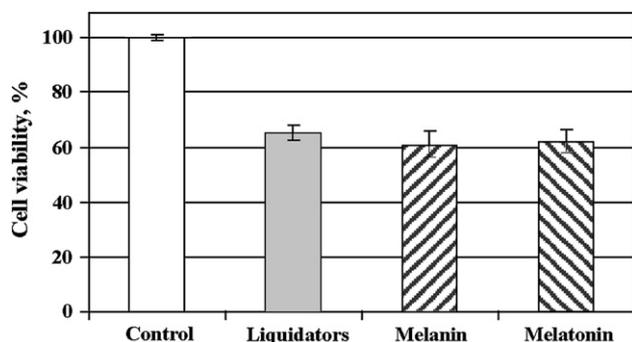


Figure 6. Average data obtained in study of the influence of melanin and melatonin on HPV-G cells treated with blood serum samples from Chernobyl liquidators.

difference could be seen between the CBA cancer-prone strain and the C57 B16 strain, which respond to radiation by inducing apoptotic pathways. Such sectoring along genetic lines could be important in human populations exposed to radiation. Because genomic instability is thought to be a “life with damage” option for irradiated cells, it was decided to compare individual patient serum samples for their ability to induce death (loss of viability in the Almar Blue assay) vs micronucleus formation (taken as an indicator of genomic instability). As can be seen from the results obtained, the viability of the cells treated with serum samples from healthy people is very close to control (nontreated). Treatment of the cells with serum samples from Chernobyl liquidators clearly reduces the viability of HPV-G cells more than 1.5 times; from $24.89 \pm 0.25 \times 10^3$ FU (control) and $24.67 \pm 0.62 \times 10^3$ FU (Healthy People) to $15.65 \pm 0.82 \times 10^3$ FU (Liquidators); $p < 0.01$ in both cases ($t = 10.79$ and 8.77 , respectively). Treatment of HPV-G cells with serum samples from residents of contaminated territories also reduces the viability of cells (average viability is $19.16 \pm 0.71 \times 10^3$ FU), but not as significantly as serum samples from liquidators [$t = 7.62$ (compared to controls) and $t = 5.84$ (compared to serums from healthy people)]. However, increased genomic instability in an individual was associated with decreased viability, suggesting that cells were not making a choice between death and genomic instability at this level.

In previous work (Mosse et al. [18]), it was shown that bystander signal may be partially reduced melanin, which has radical scavenging capacities. In this work, we also tried to prevent the damaging action of serum samples from victims of Chernobyl accident using melanin. Addition of melanin to the medium together with serum samples from Chernobyl liquidators does not have any protective effect.

To conclude, these results, using both death and genomic instability as endpoints of long-lived radiation effects, show that agents capable of causing cell death or genomic instability can persist in serum samples even 20 years after the accident. There is also preliminary evidence that acute virus

infection can increase the frequency of clastogenic effects. It should be noted that the long-term effects described are generally indicative of lethal damage during cell division at the cellular level. Cellular “effect” does not equate simply with “harm” and one role of these factors could be to eliminate cells carrying damage, thus cleansing the organism of harmful cells with potentially carcinogenic mutations.

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