Somatic Mutations at the Glycophorin A (GPA) Locus Measured in Red Cells of Chernobyl Liquidators Who Immigrated to Israel

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Glycophorin A (GPA) assays for human erythrocytes with gene expression loss and duplication phenomena (N0, NN) were carried out on 15 Chernobyl clean-up workers (liquidators) who immigrated to Israel within the preceding 5 years, 19 local Israeli controls, and 14 Russian (nonliquidator) immigrants. GPA phenotype variants in red blood cells of the 15 liquidators showed values ranging from 1 to 101 events/10^6 cells, with a mean ± SD of 25.6 ± 7.0. In comparison, the 19 Israeli controls had values ranging from 0 to 13 GPA events per 10^6 cells, with a mean ± SD of 3.9 ± 0.8. The difference was highly significant (p<0.001). Another group of 14 volunteer control subjects (nonliquidators) who had emigrated from the former Soviet Union to Israel during the past 5 years showed values ranging from 0.0 to 35.0 events per 10^6 cells, with a mean ± SD of 6.1 ± 2.7. The difference between this group and the liquidator group was significant at p<0.01. The results are compatible with past exposure to radiation in the group identified as liquidators. — Environ Health Perspect 105(Suppl 6):1451–1454 (1997)

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Introduction

On 26 April 1986 there was an explosion and fire in reactor number 4 of the Chernobyl power plant. More than 10^18 Bq of radioactive substances were released into the atmosphere, including 1.4×10^18 Ci of I-131 and other short-lived radioiodines that were dispersed by winds and precipitated with rain. People over vast areas were exposed to variable amounts of β- and γ- irradiations from the emitted radioisotopes. A subgroup of this population consisted of salvage or clean-up workers, called liquidators, sent to work during and after the accident in different jobs near and within the damaged reactor. Their total number has been estimated to be as high as 600,000. Although their exposure was supposed to have been limited to 25 cSv, the actual radiation doses that these people received is not clear.

During 1990, mass migration to Israel from the former Soviet Union (FSU) started, and by 1996 about 700,000 people had come to Israel. Roughly 20% of this number is estimated to have emigrated from radiocontaminated areas of Belarus, Russia, and Ukraine. Within this immigrant group were a limited number of clean-up workers. The presence of these people in Israel has led to the possibility of estimating the extent of past radiation exposure using various biological indicators (I).

The somatic mutation test of human erythrocytes with gene expression loss and duplication phenomena at the glycophorin A (GPA) locus (2) has been found to be a sensitive indicator of exposure to radiation in atom bomb survivors (3), individuals exposed in the Goaia accident (4), and in Chernobyl-exposed groups (5–7). In the present work, our objective was to apply the GPA somatic mutation assay to a group of Chernobyl clean-up workers who immigrated to Israel and to compare them with unexposed control subjects in Israel and immigrants who came from various areas of the FSU.

Materials and Methods

Subjects

Of 41 volunteer clean-up workers who had official papers identifying themselves as liquidators or clean-up workers at the Chernobyl nuclear power plant, only 15 were identified as having the MN blood type. There were two groups of control subjects. The first group consisted of 40 Israeli residents who had not come from FSU countries, of whom 19 had the MN blood type. The second group of 26 controls were emigrants from FSU countries to Israel who were selected randomly; of these 14 had the MN blood type.

The age range of the liquidators with blood type MN was 30 to 63 years of age (73% male), Israeli MN controls were 20 to 69 years of age (47% male), and the FSU MN controls were 27 to 64 years of age (29% male).

Methodology

Peripheral blood was obtained by venipuncture from each volunteer. Each sample consisted of 5 to 10 ml of heparinized blood. The samples were coded and each sample was tested for determination of the blood type—MM, MN, or NN. This was done using commercial anti-M and anti-N antibodies obtained from Ortho Diagnostic Systems Inc. (Raritan, NJ). Identification of the blood types was carried out according to the commercial instructions.

The flow cytometric assay for N0 and NN phenotypes was performed according to the BR6 method of Langlois et al. (8). The assay enumerates the frequency of N0 and NN phenotypes in a cell population of MN blood type. This procedure can be performed only on the 50% of the population expressing the heterozygous MN phenotype. The assay uses immunofluorescence labeling and flow cytometry to enumerate the frequency of red blood cells lacking...
expression or showing duplication of one allele. The fluorescent anti-M antibody (6A7) labeled with rhodamine, and the fluorescent anti-N labeled with fluorescein isothiocyanate (FITC) were obtained from the International Blood Group Reference Laboratory (Bristol, U.K.).

The blood was fixed and labeled with fluorescent monoclonal antibodies and analyzed by flow cytometry using a Becton-Dickinson FACSTAR (San Jose, CA) fluorescence-activated cell sorter (FACS). The time taken from drawing the blood to FACS analysis was 5 to 14 days, during which time the whole blood samples were kept at 4°C.

The results are the sum of both NØ and NN phenotypes.

Statistical Analysis
Comparison was made between the results obtained from the liquidators and the two control groups using the Student’s t-test and the Mann-Whitney test.

Results
The results of the study are summarized in Figure 1. The 19 Israeli controls showed tight distribution within the range of 0 to 13 per 10⁶ cells. The mean ± SD was 3.9 ± 0.8. In contrast, the 15 liquidators showed values ranging from 1 to 101 per 10⁶ cells, with a mean ± SD of 25.5 ± 7.0. The difference was highly significant at p < 0.001.

To determine whether the difference was due to radiation exposure at Chernobyl or to differences between the people living in areas of the FSU and to those in Israel who did not come from the FSU, a second group of controls was obtained. The 14 MN control individuals in the latter group had values ranging from 0.0 to 35.0, with a mean ± SD of 6.1 ± 2.7 per 10⁶ cells. Compared with the liquidator group, the difference was also significant at p < 0.01.

Discussion
Various biological methods have been developed to estimate human exposure to ionizing radiation. These are based mainly on chromosomal damage and mutagenic changes at the cellular level (1). One sensitive technique, originally described by Langlois et al. (2,9), measures the frequency of red cells carrying a mutation at the GPA locus in red cells, presumably because of mutation of erythroid precursor cells. In this technique, fluorescent monoclonal antibodies specific for group M or N alleles are used to identify the cell that expresses only one allele. It was found that the normal frequency of variant cells missing the M or N allele is about 1 in 10⁵. Langlois et al. (8) also described an improved version of this method using a single-beam cytometer (BRG), which led to improvements in separation of normal and mutant phenotypic cells.

The GPA gene is present in single copy in the genome of chromosome 4 (10,11). The protein coding region of the GPA gene is 453 nucleotides in length and consists of 7 exons and 6 introns extending over 40 kb. The GPA gene occurs as two common alleles, GPA™ and GPA÷N, that are responsible for MN blood groups. GPA can carry either blood group M or N antigens, as determined by the amino acid sequence at residue 1 and residue 5 of the mature glycoprotein that is Ser¹/Gly³ for M and Leu¹/Glu³ for N (12,13). These antigens differ by only 2 amino acids out of 131. The two alleles are co-dominantly expressed as MN heterozygotes and approximately equally represented in the human population, so that heterozygotes represent about 50% of all individuals. Hence, only 50% of the population can be analyzed because they are heterologous at this locus (14).

Exposure to mutagens such as ionizing radiation can cause damage to precursor erythroid cells of the bone marrow. Grant and Bigbee (15) reported that the GPA assay distinguishes between two independent classes of variant cells. In one class, the expression of one allelic form of GPA surface protein is lost, and in the second, in addition to allelic loss the cells express the remaining homolog at twice the level of the heterozygote. Thus, in individuals having MN blood type, the GPA mutants are recognized on rare red blood cell variants as either the simple loss of M or N expression (NØ, MØ), termed hemizygous mutations, or loss of expression of one of the alleles, with double expression of the other (NN, MM, with the mutation being called homozygous). The natural frequency of variants was estimated to be 7.1 ± 5.6 per 10⁶ cells (16), but naturally increases with age (17) and with smoking (18) have been observed.

The GPA assay was used to show elevated levels of red cell variants due to somatic mutation in human groups exposed to radiation such as that from Hiroshima and Nagasaki (2,3,19–22) and from the Goiania radiation exposure accident (4). Increases in the frequency of variants have been found after exposure to mutagenic drugs (23) as well as in cancer-susceptible patients with ataxia telangiectasia (24) and Bloom’s syndrome (25,26). It has been suggested, therefore, that the GPA somatic mutation assay has value for the assessment of cancer risk (20,27).

Jensen et al. (5) evaluated clean-up workers from Russia and Ukraine and found in 115 individuals a significant increase of NØ red cells corresponding to the estimated radiation exposure. The increased frequency of NØ variants was stable in 10 donors over a 7-year period. Somatic mutation analyses using the GPA assay were also carried out by Bigbee et al. (6,7) on Estonian clean-up workers exposed to ionizing radiations as a result of the Chernobyl accident. However, in the latter group, the frequency of variant erythrocytes was low, suggesting that the dose to the workers did not exceed 10 cGy, probably too low a dose to result in detectable damage to erythroid stem cells.

The natural frequency of NØ red cells was reported by Jensen et al. to be 7.1 ± 5.6 per 10⁶ cells in U.S. residents not exposed to radiation (16) and 6.6 ± 4.7 per 10⁶ cells (5) for unexposed Ukrainian and Russian subjects. These values compare well with those for our Russian control group (6.1 ± 2.7 per 10⁶ cells), whereas the frequency we observed in Israeli residents was somewhat lower at 3.0 ± 0.8 per 10⁶ cells though the difference was not statistically significant.

In this study we have used the GPA assay to measure the frequency of mutations in red cells of salvage workers (liquidators) of the Chernobyl accident who had immigrated to Israel. Our measurements, which were carried out 9 to 10 years after the accident, showed significant elevation in the number of variant red cells of liquidators compared to the number observed among Israeli and FSU immigrant control subjects.
These results suggest the presence of clones of phenotypically variant red cells in which the N antigen is missing or duplicated, presumably an indication of mutated red cell erythroid precursor cells in the bone marrow.

Kyoizumi et al. (20) reported a slightly greater frequency of GPA NO cells in males than in females (by ~10%) in unexposed survivors in Hiroshima and Nagasaki, but no effect of sex on dose response was observed. Although control groups contained a lower proportion of males, compared to the liquidator group, the marked difference reported between liquidators and controls is not explainable on the basis of the natural male-to-female difference in NO frequency.

The effects noted in this study may be attributable to radiation exposure on the part of the liquidators during the course of their activities at the Chernobyl site 9 to 10 years earlier. Therefore, it was important to compare these effects with those of unexposed persons who had emigrated from the FSU as well as with those of residents of Israel. The fact that the emigrant FSU control subjects did not show the effect on NO frequency as seen in liquidators does not support the possibility that mutagenic agents (apart from radiation) in their previous residential or working environments caused these changes.

A rough estimation of radiation exposure dose based on the results of the GPA somatic mutation assay may be obtained by the method of Straume et al. (28) who used the following equation:

\[ H = \frac{[N_{0w} - N_{0b}]/\text{slope of standard curve}] \times Q}{N} \]

where

- \( H \) = dose equivalent in Sv,
- \( N_{0w} \) = frequency of NO variants in the radiation exposed person,
- \( N_{0b} \) = frequency of NO variants in unexposed controls,
- \( Q \) = quality factor

The slope NO per Gy was estimated by Straume et al. (28) on the basis of results obtained from Hiroshima atomic bomb survivors and from Goiania radiation accident victims. Values for Hiroshima survivors led to slope values of \( 48 \times 10^{-6} \) per Gy from data of Langlois et al. (3), and independent GPA measurements by Kyoizumi et al. (19) provided NO values for Hiroshima survivors of \( 40 \times 10^{-6} \) per Gy. These values were of course based on radiation exposures that occurred about 40 years before the GPA measurements were made.

More recent exposures from the 1987 Goiania radiation accident were tested by Straume et al. (4). In this accident, Cs-137 exposure was received over a period of about 2 weeks. Using dose estimates of Ramalho et al. (29), the slope was estimated to be \( 12.8 \times 10^{-6} \) per Gy.

The Chernobyl liquidators were exposed primarily to external 'shine' radiation and inhalation of emitted radionuclides, primarily \( \beta \) and \( \gamma \), although some \( \alpha \) emitters were also present. Therefore, the quality factor can be assumed to be equal to one.

Control variant cell frequencies (from unirradiated Israeli residents) averaged 3.9 \pm 0.8 per 106 cells, whereas 15 liquidators showed values averaging 25.5 \pm 7.0 per 106 cells. Using the slope based on the Goiania data, a rough estimate of 1.7 Sv mean exposure dose is made. Using the slope from Hiroshima, the estimate is 0.5 Sv. If the value of 10 events per 106 cells is used for background frequency of variant cells, then the minimal dose is 15.5/48 = 0.32 Sv. Values in the range 0.3 to 1.7 Sv are consistent with average doses of 0.51 (acute) and 0.96 Gy (chronic) estimated by Lucas (30) using fluorescence in situ hybridization for 12 exposed Chernobyl liquidators without medical symptoms. Thus, these rough and rather tentative estimates suggest that radiation exposures occurred above 0.25 Sv, which was the provisional upper limit specified at the time of the accident.

Nevertheless, it must be pointed out the nature of the exposures at Hiroshima differed considerably from those of Goiania, and both differed in terms of intensity, duration, source of radiation, and time of GPA analysis from the exposure incurred by the Chernobyl liquidators. Therefore, calculations of dose to liquidators based on GPA analyses of atom bomb survivors and Goiania findings are not necessarily reliable.

The difference between these results, which support those of Jensen et al. (5), and the low values found in the studies by Bigbee et al. (6,7) with groups of liquidators from Baltic countries is of interest, and may indicate that radiation exposures to different liquidator groups were not uniform. Correlating the results of biological indicators of exposure with dose reconstruction estimates probably would be useful.

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