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PERSPECTIVE ARTICLE

Complex Chromosome Aberrations Persist in Individuals Many Years After Occupational Exposure to Densely Ionizing Radiation: An mFISH Study

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Long-lived, sensitive, and specific biomarkers of particular mutagenic agents are much sought after and potentially have broad applications in the fields of cancer biology, epidemiology, and prevention. Many clastogens induce a spectrum of chromosome aberrations, and some of them can be exploited as biomarkers of exposure. Densely ionizing radiation, for example, alpha particle radiation (from radon or plutonium) and neutron radiation, preferentially induces complex chromosome aberrations, which can be detected by the 24-color multifluor fluorescence in situ hybridization (mFISH) technique. We report the detection and quantification of stable complex chromosome aberrations in lymphocytes of healthy former nuclear-weapons workers, who were exposed many years ago to plutonium, gamma rays, or both, at the Mayak weapons complex in Russia. We analyzed peripheral-blood lymphocytes from these individuals for the presence of persistent complex chromosome aberrations. A significantly elevated frequency of complex chromosome translocations was detected in the highly exposed plutonium workers but not in the group exposed only to high doses of gamma radiation. No such differences were found for simple chromosomal aberrations. The results suggest that stable complex chromosomal translocations represent a long-lived, quantitative, low-background biomarker of densely ionizing radiation for human populations exposed many years ago.

INTRODUCTION

Ionizing radiation is highly efficient in producing chromosome aberrations as a consequence of misrejoining of induced DNA double-strand breaks (Savage, 1998). Unstable (dicentrics, acentric fragments, and rings) and stable (translocations) aberrations are the two major classes of chromosomal alterations induced by irradiation of cells in the G0 or G1 stage of the cell cycle. In vivo and in vitro studies using mouse models and human blood lymphocytes to determine the persistence of chromosome aberrations following radiation exposure (Lucas et al., 1992a, 1992b; Hande et al., 1996; Hande and Natarajan, 1998) have demonstrated that stable chromosome translocations produced by ionizing radiation persist long after exposure. This opens up the possibility of using chromosome aberrations as a biomarker of radiation damage or as a biodosimeter of radiation exposure many years after medical, accidental, or occupational exposure (Tucker, 2001).

Chromosome aberrations involving three or more breaks in two or more chromosomes are defined as complex (Savage and Simpson, 1994). In vitro, they have been shown to be preferentially induced after exposure to low doses of densely ionizing radiation such as that of alpha particles or neutrons, compared with sparsely ionizing radiation such as that of X-rays (Griffin et al., 1995; Testard et al., 1997; Knehr et al., 1999; Anderson et al., 2000, 2002, 2003; Boei et al., 2001; Moquet et al., 2001). This preferential production of complex aberrations by densely ionizing radiation is related to the unique energy deposition patterns produced by densely ionizing radiation, which produces highly localized multiple DNA damage at the chromosomal level (Brenner and Ward, 1992;

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Prise et al., 2001; Anderson et al., 2002). Almost all other mutagens, such as chemicals, X-rays, and general aging processes, produce DNA damage whose spatial distribution is far more homogeneous. On the basis of the results of these in vitro studies, several authors have suggested that complex chromosome aberrations might be useful as a fingerprint of past exposure to densely ionizing radiation (Anderson et al., 2000; Boei et al., 2001). In the present study, we investigated this supposition in a human population exposed some years ago to densely and sparsely ionizing radiation.

All the in vitro studies on complex chromosome aberrations mentioned above used fluorescence in situ hybridization (FISH) technology, in which whole chromosomes are painted with a unique color. The exposed human populations that have been most extensively studied using FISH are A-bomb survivors and Chernobyl liquidators (Nakano et al., 2001; Jones et al., 2002). Most human population studies using FISH examined only a few chromosomes, using two- or three-color FISH to detect chromosome translocations (Tucker, 2001). This is clearly suboptimal for the study of complex chromosome aberrations, which involve multiple chromosomes. The development (Speicher et al., 1996) of multifluor FISH (mFISH), in which all 22 autosomes plus the two sex chromosomes are painted in different colors to determine chromosome alterations, has greatly improved our ability to identify chromosome aberrations in general and complex aberrations in particular (Anderson et al., 2003; Wu et al., 2003). For example, using the 24-color mFISH technique, Wu et al. (2003) were able to detect an increased proportion of complex aberrations in human fibroblasts after in vitro exposure to densely ionizing radiation, whereas this effect was not seen using two- or three-color FISH (Durante et al., 2004).

We recently reported the first use of the mFISH technique on a previously exposed human population (Hande et al., 2003) in order to study chromosome aberrations in former reactor workers at the Mayak reactor facility, Ozyorsk, Russia. In that study, the mFISH technique was used to measure simple translocations. In the present study, we used the 24-color mFISH technique to measure complex chromosome aberrations in the same population of individuals occupationally exposed many years earlier to sparsely and densely ionizing radiation.

These former nuclear-weapons workers at the Mayak Production Association, Ozyorsk, Russia, provide a unique opportunity to analyze the genetic risk of ionizing radiation to humans (Anspaugh et al., 2002). Individuals in the study population were occupationally exposed to ionizing radiation from 1949 on. At Mayak, the plutonium workers were exposed both to densely ionizing alpha particles as a consequence of plutonium inhalation and to sparsely ionizing gamma rays, whereas the reactor workers were exposed to gamma rays but not to plutonium.

We recently showed that even many years after occupational exposure, a significant fraction of the cells of healthy Mayak plutonium workers contained large intrachromosomal rearrangements (Hande et al., 2003; Mitchell et al., 2004). In the present article, we report detecting persistent complex chromosome translocations in peripheral-blood lymphocytes of Mayak plutonium workers but not in gamma-ray-exposed reactor workers, making complex chromosome translocations potentially an additional biomarker of plutonium exposure in humans.

MATERIALS AND METHODS

Population Studied

The studied individuals had been occupationally exposed to ionizing radiation from 1949 onward at the Mayak Production Association near Ozyorsk, Russia (Anspaugh et al., 2002). Both plutonium and reactor workers were exposed to sparsely ionizing gamma rays, but only plutonium workers suffered exposure to densely ionizing alpha particles, which was a consequence of their inhalation of plutonium. Both groups also were exposed to a variety of chemical mutagens. The Mayak workers have been reported to have an increased risk of lung (Kreisheimer et al., 2000), liver (Gilbert et al., 2000), and bone (Koshurnikova et al., 2000) cancer, although all the subjects in the present study are apparently healthy.

The database of healthy former workers living near the Mayak plant in 2001 for whom both gamma-ray and plutonium dosimetry were available numbered just over 4,000 individuals. This group was further categorized by exposure (plutonium or gamma ray) and by when occupational exposure began. Our pilot study design was to consider individuals in the group with the earliest start of occupational exposure (exposure that began before 1955) and to compare individuals in the high-plutonium-dose group [>0.4 Gy Pu dose to bone marrow] with individuals in the zero plutonium/high γ-ray dose group [>1.5 Gy γ dose to bone marrow]. These criteria resulted in choosing

TABLE 1. Dosimetry Details of the Three Groups of Mayak Workers with Occupational Exposure to Radiation (Hande et al., 2003)

	Highly exposed plutonium workers ($n = 11$)	Reactor workers (zero plutonium) ($n = 11$)	Moderately exposed plutonium workers ($n = 4$)
Mean start/end dates of occupational exposure	1951/1971	1949/1977	1980/1989
Mean age (range) at time of sampling	75 (68–82)	74 (67–82)	66 (62–77)
Mean plutonium dose (range) to bone marrow (Gy)	1.1 (0.4–2.1)	0	0.19 (0.11–0.33)
Mean gamma-ray dose (range) to bone marrow (Gy)	1.5 (0–3.1)	2.3 (1.5–3.8)	0.19 (0.07–0.31)

11 individuals from each group, all of whom agreed to participate in the study.

A small group ($n = 4$) of workers who received only moderate plutonium exposure and an unexposed control group ($n = 5$) of healthy workers also were assessed. Mean doses and start/end dates of occupational exposure for each of these groups are given in Table 1.

The study was approved by the appropriate institutional review boards, and informed consent was given by each subject.

Chromosome Preparations

Blood samples were collected from the 31 individuals during 2001, and chromosome aberrations in peripheral-blood lymphocytes were scored. Metaphase preparations were made at the Southern Urals Biological Institute in Ozyorsk, Russia, using standard protocols (Burak et al., 2001). In this study, lymphocyte cultures were initiated 4–5 days after the blood was drawn and were incubated for 48 hr before metaphase slide preparation. mFISH (Speicher et al., 1996) and aberration scoring were subsequently carried out in New York. For the mFISH analysis to detect complex chromosomal aberrations, between 110 and 160 metaphase spreads for each individual were scored.

mFISH Assay

Chromosome paints were obtained from MetaSystems GmbH, Germany. Microscopic analysis was performed using an Axioplan II imaging microscope (Carl Zeiss, Jena, Germany) with an HBO-103 mercury lamp and filter sets for FITC, Cy3.5, Texas Red, Cy5, Aqua, and DAPI. Images were captured, processed, and analyzed using ISIS mBAND/mFISH imaging software (MetaSystems, Altlussheim, Germany). In the mFISH technique, each chromosome (1–22 and X and Y) is painted a different color, using combinatorial labeling, so that any interchromosomal translocations are observed as color junctions on individual chromosomes. Painting every chromosome a different color significantly improves the precision and accuracy of translocation scoring (Greulich et al., 2000), compared with the standard, partial-genome FISH labeling (Tucker, 2001).

Worker Dosimetry

Individual estimates were made of the plutonium and gamma-ray doses to the bone marrow of each individual studied. Currently, plutonium dosimetry estimates are available for about 8,700 individuals and gamma ray dosimetry for about 10,000 (Romanov et al., 2002), of whom about 4,000 are alive, healthy, and living in the area.

Plutonium exposure is determined on the basis of urine sample measurements (Khokhryakov et al., 2000); the details are summarized by Hande et al. (2003). It is important to note that, unlike exposure to gamma rays, which terminated with the end of an individual's employment at Mayak, some of the plutonium exposure occurred subsequently because of long-term retention of a fraction of the plutonium intake. For the plutonium workers studied, an estimated average of 50% of the bone marrow plutonium dose was deposited after 1983, 25% was deposited after 1993, and 8% was deposited after 1998.

Data on gamma-ray exposure, which came from external sources, is based primarily on film badge data (Romanov et al., 2002); details were also summarized by Hande et al. (2003). The reactor workers also had very low exposure to neutron radiation: the maximum neutron dose to the bone marrow was estimated to be less than 0.3% of the gamma-ray dose.

Dosimetry details for each group in the present study were published earlier (Hande et al., 2003) and are summarized in Table 1.

Statistical Methods

The results are expressed as mean ± standard deviation. Statistical comparisons between groups were made using the exact Wilcoxon–Mann–Whitney test (Lehmann, 1975), which involved the direct comparison of individual aberration frequencies in two groups.

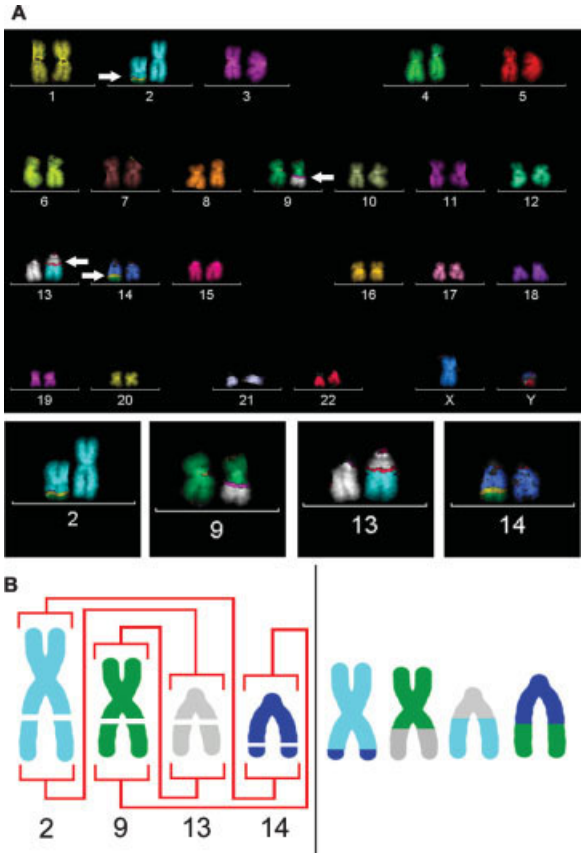

Figure 1. A stable complex chromosome translocation in a Mayak plutonium worker, detected with mFISH: (A) Chromosomes involved in this aberration are indicated by arrows; a complex chromosome translocation is shown involving four chromosomes: 2, 9, 13, and 14. (B) Schematic description of this same complex aberration, which was produced by misrejoining of breaks on four chromosomes.

Significance was assessed on the basis of Bonferroni-adjusted levels.

RESULTS

Figures 1 and 2 show two typical complex translocation aberrations, detected in this study with mFISH. In the mFISH technique, all chromosomes are painted different colors, enabling visualization of chromosome translocations as color junctions on particular chromosomes. On the basis of these results, the pattern of initial chromosome breaks that resulted in a complex aberration can generally be reconstructed (Levy et al., 2004), as shown in Figures 1B and 2B.

The overall quantitative results are given in Table 2 and illustrated in Figure 3. The frequency of complex chromosome aberrations in the highly exposed plutonium workers was 2.9% ± 0.4%, significantly higher than that in the reactor workers with zero plutonium exposure (0.2% ± 0.1%), as well as in the moderately exposed plutonium workers (0.2% ± 0.1%) and the zero-dose controls (0%).

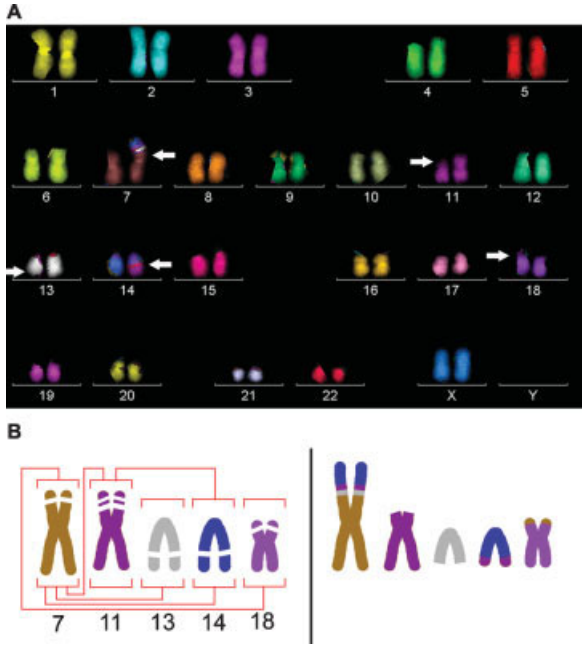

Figure 2. A stable complex chromosome translocation in a Mayak plutonium worker, detected with mFISH. (A) Chromosomes involved in this aberration are indicated by arrows; a complex chromosome translocation is shown involving five chromosomes: 7, 11, 13, 14, and 18. (B) Schematic representation of this same translocation, which was produced by misrejoining of breaks on five chromosomes.

The bottom line is that the frequency of complex aberrations was significantly higher in the highly exposed Pu workers than in the reactor workers (who received only high doses of sparsely ionizing gamma rays); in contrast, the frequency of simple translocation aberrations was comparable in these two groups. It is also pertinent that in the zero-dose controls, the frequency of complex aberrations was 0%, significantly lower than that of simple translocations (0.7% ± 0.3%).

Individual details about the chromosomal translocation yields in the 15 plutonium workers are provided in Table 3 and Figure 4. There was a significant correlation ($P < 0.03$) between the estimated plutonium dose to the bone marrow and the yield of complex aberrations (Pearson correlation coefficient, $r = 0.57$).

Finally, we note that the complex chromosome aberrations detected in the present study were strikingly similar to those found in some tumor cells (Brizard et al., 2004; Muller et al., 2004).

DISCUSSION

Potential Significance of Duration of Exposure

Unlike the gamma-ray exposure, which terminated with the end of an individual's employment at Mayak, a fraction of the plutonium exposure

TABLE 2. Measured Yields of Complex and Simple Translocations in the Four Groups Studied

	Measured chromosome aberrations/cells examined			
	Highly exposed Pu workers ($n = 11$)	Reactor workers (zero Pu intake) ($n = 11$)	Moderately exposed Pu workers ($n = 4$)	Controls ($n = 5$)
Interchromosomal complex translocations	41/1.414[a] = 2.9% ± 0.4%	3/1.424 = 0.21% ± 0.1%	1/444 = 0.23% ± 0.2%	0/582 = 0%
Interchromosomal simple translocations	82/1.414[b] = 5.8% ± 0.6%	67/1.424 = 4.7% ± 0.6%	10/444 = 2.2% ± 0.7%	4/582 = 0.7% ± 0.3%

Complex translocations involve more than two breaks on two or more chromosomes. Simple translocations involve two breaks on any heterologous pair of chromosomes.

[a]Significantly greater ($P < 0.005$, Wilcoxon–Mann–Whitney test, Bonferroni adjusted), compared with reactor workers, with moderately exposed Pu workers, and with controls.

[b]7/1.414 unstable interchromosomal aberrations (dicentrics) were also detected in this group.

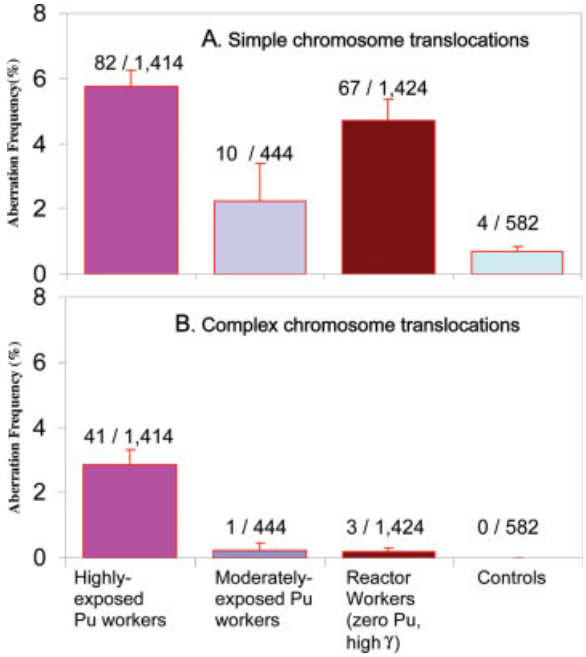

Figure 3. Measured yields of stable chromosomal translocations in peripheral-blood lymphocytes of highly exposed plutonium workers, moderately exposed plutonium workers, reactor workers, and unexposed control individuals. Shown are the number of aberrations found/number of metaphase cells examined. (A) Simple chromosome translocations between heterologous pair of chromosomes. (B) Complex chromosome translocations, defined as resulting from three or more breaks involving two or more chromosomes. Note the similar yields of simple aberrations in the highly exposed Pu workers versus the reactor workers (who received only high doses of sparsely ionizing gamma rays). By contrast, the highly exposed Pu workers had a significantly higher yield of complex aberrations than did the reactor workers (who received no plutonium exposure) and those in the control group.

occurred subsequently because of long-term retention of a fraction of the plutonium intake (Khokhryakov et al., 2002). For these plutonium workers, even though the mean starting/ending dates of their occupational exposure to plutonium were 1951/1971, an average of 50% of the current plutonium dose in their bone marrow had been deposited after 1983, 25% had been deposited after 1993, and 8% had been deposited after 1998.

It is important, therefore, to consider whether the excess number of complex aberrations in the plutonium workers could be related to their exposure having occurred more recently than that of most of the reactor workers; this appears unlikely, based on the following considerations. The observed yield of unstable (nontransmissible) dicentric aberrations in the plutonium workers was about 9% of the stable simple translocation yield (see Table 2 footnote). These dicentrics were initially produced with about the same frequency as the apparently simple translocations (Straume and Lucas, 1993; Lucas et al., 1996), but because of their relatively rapid disappearance (Ramalho et al., 1995; IAEA, 2001), these dicentrics must have been produced within the past 3 years, that is, by about 8% of the total plutonium dose. Thus, it is reasonable to conclude that about 9% of the measured stable aberration yield in the plutonium workers was formed within the past 3 years by about 8% of the total plutonium dose, with the remainder of the stable aberrations having been produced earlier. Consequently, it is unlikely that the higher observed yield of stable complex aberrations in the plutonium workers could simply be a result of their having been produced much more recently than those in the reactor workers.

Long-Term Stability of Complex Chromosome Translocations

Despite the complex nature of the chromosome aberrations measured here (see, for example, Figs. 1 and 2), the comparatively long period that had elapsed since the occupational exposure of the plutonium workers, and their healthy status, complex chromosome aberrations were detected in all the

TABLE 3. Individual Yields of Stable Complex Chromosome Translocations Measured in the Mayak Plutonium Workers

Subject[a]	Plutonium dose to bone marrow (Gy)	No. of cells examined	No. of complex chromosome translocations
1	2.08	136	4
2	2.00	126	2
3	1.27	108	4
4	1.21	131	5
5	1.13	144	9
6	1.02	106	3
7	0.94	135	5
8	0.89	129	3
9	0.77	120	2
10	0.64	153	3
11	0.44	126	1
12	0.33	110	0
13	0.17	113	0
14	0.14	110	0
15	0.11	111	1

[a]Subjects 1–11 made up the group of highly exposed Pu workers; subjects 12–15 made up the group of moderately exposed Pu workers.

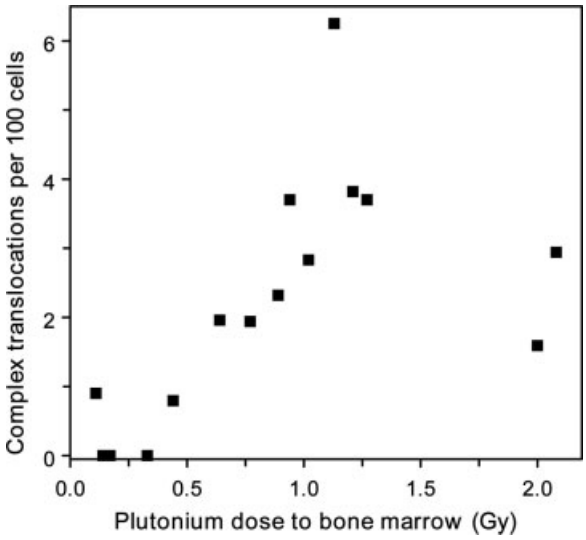

Figure 4. Scatter plot of complex translocation frequency (per 100 cells examined) versus estimated plutonium dose to the bone marrow. A significant correlation ($P < 0.03$) was found between the plutonium dose and the yield of complex aberrations (Pearson correlation coefficient, $r = 0.57$).

highly exposed plutonium workers that we examined. The overall frequency was 2.9 ± 0.4 complex aberrations per 100 cells examined.

Biodosimetry estimates of past radiation exposure by determining the frequency of chromosome aberrations require the measurement of transmissible aberrations (those capable of passing through mitotic divisions in the hemopoietic cell reproductive compartments). There is considerable debate about the stability of radiation-induced transmissible chromosome aberrations. Some authors (Salassidis et al., 1995; Stephan and Pressl, 1997; Lloyd et al., 1998; Lindholm and Edwards, 2004) have reported that simple translocations, at least, are highly stable over many years, whereas others (Matsumoto et al., 1998; Bauchinger et al., 2001a, 2001b) have suggested that there is a slow loss over time.

A priori, it might have been expected that the complex aberrations measured in the present study would have a rather lower transmissibility and thus a more rapid loss over time than would simple translocations. However, the results shown here indicate that the radiation workers, despite their healthy and long-lived status, maintained a considerable burden of stable complex chromosomal aberrations many years after occupational exposure. Thus, although we do not know the initial induction rate of simple versus complex aberrations in this population, it is clear that the complex aberrations were not being lost at a much greater rate than were simple translocations.

Specificity of Complex Chromosome Aberrations

The data in Figure 3 show that simple chromosomal translocations, although a proven biomarker for past exposure to ionizing radiation in general (Tucker, 2001), are produced efficiently both by sparsely and by densely ionizing radiation. In contrast, the yield of complex chromosomal aberrations was significantly greater in the workers exposed to plutonium than in the reactor workers, who were exposed only to high gamma-ray doses. Indeed, the yield of complex chromosomal aberrations in the reactor workers was not significantly elevated compared with the zero-dose controls.

Thus, these measurements suggest that complex chromosome aberrations may be a useful biomarker of densely ionizing radiation exposure in a human population exposed many years earlier. These results in a human population confirm earlier in vitro–based studies (Griffin et al., 1995; Testard et al., 1997; Knehr et al., 1999; Anderson et al., 2000, 2002, 2003; Boei et al., 2001; Moquet et al., 2001), as well as earlier suggestions (Anderson et al., 2000; Boei et al., 2001) that complex chromosomal aberrations have the potential to be a specific biomarker for past exposure to densely ionizing radiation.

Possible Confounding from Exposure to Chemical Mutagens

On the basis of information about corresponding U.S. plutonium workers (Brandom et al., 1990),

some workers in the present study are likely to have been exposed to chemical mutagens—some combination of perchloroethylene, beryllium, carbon tetrachloride, benzene, and trichloroethylene. As in the U.S. database, however, no individual or quantitative data are available in the Mayak worker dosimetry database.

These chemicals initially would be expected to produce predominantly chromatid-type damage that after cell division could result in chromosome aberrations. As discussed above, the spatial distribution within a cell of DNA damage from chemical exposure is far more homogeneous than is that from densely ionizing radiation and would be expected to resemble much more that from X- or gamma-ray exposure. Chromatid damage from X-rays has been shown to contain a much smaller proportion of complex chromatid damage compared with alpha particles (Griffin et al., 1994), and so, on the basis of the expected spatial distribution of DNA damage from the chemicals to which the Mayak workers may have been exposed, chemically induced damage would be expected to show aberration patterns similar to those of X-rays, rather than those of alpha particles.

Mechanistic Background

It is important to note that the preferential production of complex chromosome translocations (e.g., Figs. 1 and 2) by densely ionizing radiation was consistent with mechanistic expectations. The mechanistic background stems from two phenomena: (1) alpha particles and neutrons produce highly localized spatial clustering of DNA double-strand breaks (DSBs) at the chromosomal level (Brenner and Ward, 1992; Prise et al., 2001; Anderson et al., 2002); almost all other mutagens, such as chemicals, X-rays, gamma rays, and general aging processes, produce far more homogeneous spatial distributions of DSBs within the genome. (2) DSB interactions to form chromosome aberrations are characterized by strong spatial proximity effects (Sachs et al., 1997), such that breaks that are spatially closer to one another are more likely to result in chromosome aberrations. Given these two observations, it follows that the number of breaks that take part in a single reaction would be expected to be larger after densely ionizing radiation damage than after exposure to X-rays, gamma rays, chemical mutagens, or aging processes. Given that complex aberrations are defined as those involving three or more breaks (Savage and Simpson, 1994), it follows that alpha particles and neutrons would be expected preferentially to produce these types of aberrations.

Complex Chromosome Aberrations as a Potential Practical Biomarker

As illustrated in Figure 3, the frequency of complex chromosomal aberrations is significantly correlated with the plutonium dose to the bone marrow ($P < 0.03$). Thus, complex chromosomal aberrations have the potential to be used for quantitative dose reconstruction of densely ionizing radiation exposure, such as a radiological terrorist incident involving plutonium (Durante and Manti, 2002) or americium, domestic exposure to radon (Alavanja, 2002), or flight personnel exposed to neutron radiation (Blettner et al., 1998). In each of these situations, low-dose epidemiologic risk estimation, in particular of low-dose cancer risk (Brenner et al., 2003), is strongly hindered by current limitations in estimating past radiation exposure on an individual basis.

There is also good evidence from the control results that the background frequency of complex chromosomal aberrations in an unexposed population is low, significantly lower than the background frequency of simple chromosomal aberrations. This is not unexpected in that background aberrations are unlikely to be the result of densely ionizing radiation, and low doses/dose rates of other mutagens and endogenous processes would be expected primarily to produce simple aberrations (Loucas et al., 2004). Low background aberration frequency is important for dose reconstruction of low doses of radiation, in that it determines the minimum dose that can be reconstructed from measured data (Tucker, 2001).

In using complex chromosome aberrations as a biomarker of past exposure to densely ionizing radiation, it is highly advantageous—and perhaps essential—to use 24-color FISH to detect unequivocally the chromosome aberrations involved in order to cover all chromosomes (chromosomes 1–22 plus the X and Y chromosomes) for interchromosomal aberrations. Multifluor FISH enables each induced aberration to be categorized in its entirety and also offers insight into the mechanisms by which complex aberrations are formed (Levy et al., 2004).

Complex Aberrations Versus Intrachromosomal Aberrations as Biomarkers of Past Exposure to Densely Ionizing Radiation

We have described here a biomarker for densely ionizing radiation—complex chromosome aberra-

tions—that is both specific and long-lived. In previous reports (Hande et al., 2003; Mitchell et al., 2004), we also reported on the use of the mBAND technique (Chudoba et al., 1999) for measuring intrachromosomal aberrations (intra- or interarm aberrations within single chromosomes) in the same populations of Mayak workers; these intrachromosomal aberrations also were demonstrated to be a mechanistically based, sensitive, specific, and long-lived biomarker of past exposure to densely ionizing radiation (Hande et al., 2003; Mitchell et al., 2004).

Which is a more practical biomarker? Both biodosimetry assays have their advantages: It is somewhat easier to score mBAND intrachromosomal aberrations, compared with complex aberrations visualized with the mFISH technique. In addition, the frequency of intrachromosomal aberrations after exposure to densely ionizing radiation is much higher [30–50 per 100 cells (Hande et al., 2003; Mitchell et al., 2004)] than that of complex chromosomal aberrations (~3 per 100 cells; see Table 2). On the other hand, if only one or two chromosomes are assayed, as is typical with the mBAND technique, then the actual measured frequency of intrachromosomal aberrations is only a few percent, comparable to that of complex aberrations. However, recent developments with arm-specific mFISH (Karhu et al., 2001), now commercially available, suggest it is now practical to assay intrachromosomal interarm aberrations (pericentric inversions) simultaneously in all chromosomes.

CONCLUSIONS

The cancer risks associated with low levels of radiation are difficult to quantify (Brenner et al., 2003). Low-dose radiation epidemiology has been much hampered by the lack of sensitive, specific, long-lived biomarkers of past radiation exposure. Clearly, for a biomarker of past or chronic exposure to be useful, it must be stable and capable of being transmitted to daughter cells. In the present study, a recently developed chromosome-painting technique was used to measure stable complex chromosome aberrations in lymphocytes of healthy former nuclear-weapons workers in Russia who were exposed many years ago to plutonium, gamma rays, and other mutagens. The results suggest that stable complex chromosome translocations represent a sensitive, specific, long-lived, quantitative, low-background biomarker of densely ionizing radiation exposure in human populations exposed many years ago. The data from our measurement of intrachromosomal aberrations in the same population, first, in our previous studies (Hande et al., 2003; Mitchell et al., 2004), using the mBAND chromosome-banding technique and then, in the present study, using the mFISH technique, show that modern FISH techniques have the potential significantly to increase the power of radiation epidemiological studies involving densely ionizing radiation.

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