

MUT 04946

## A cytogenetic follow-up study of the victims of a radiation accident in Goiania (Brazil)

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(Received 29 August 1990)

(Accepted 17 September 1990)

**Keywords:** Gamma radiation; Goiania (Brazil); Cytogenetic follow-up

### Summary

A radiation accident involving a cesium-137 therapy source occurred in Goiania (Brazil) in September 1987, in which more than 50 individuals were exposed to moderate to high doses (0.2–7 Gy) of  $\gamma$ -radiation. A cytogenetic technique (i.e., frequencies of dicentrics and rings in peripheral lymphocytes) was employed to estimate the absorbed radiation dose. The follow-up study extending over more than 1 year indicated a decline in the frequencies of dicentrics in the lymphocytes.

Using chromosome-specific biotinylated library probes for chromosomes 1, 2, 8 and 19, we studied the frequencies of chromosomal translocations and deletions and the incidence of aneuploidy in the lymphocytes of exposed individuals. In some individuals there was a significant increase in the frequency of translocations and aneuploidy. In other experiments, in which the frequencies of HPRT mutations were determined in lymphocytes using the BrdU-labeling method, some individuals showed an increase (from about 2- to 50-fold) in mutant frequencies.

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In a radiation accident (involving a 1350-Ci, cesium-137 source) which occurred in Goiania (Brazil) in September 1987, more than 50 individuals were exposed to moderate to high doses of  $\gamma$ -irradiation (IAEA, 1988). Biological dosimetry based on frequencies of dicentrics and rings in peripheral lymphocytes was used to estimate the absorbed dose immediately following the discovery of the accident, which helped in the initial

clinical management of the patients (Ramalho et al., 1988). Cytogenetic follow-up studies have been carried out at different time intervals following the accident (Ramalho et al., 1990); these demonstrated a decrease or increase in the frequency of dicentrics with time.

We studied the frequency of hypoxanthine guanine phosphoribosyltransferase-deficient (HPRT<sup>-</sup>) mutants and chromosome aberrations in peripheral blood lymphocytes of some of these patients several months after the accident. Non-radioactive in situ hybridization using DNA probes for chromosomes 1, 2, 8 and 19 was employed to detect translocations, deletions and

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numerical aberrations involving these chromosomes. To detect HPRT mutants in lymphocytes, the bromodeoxyuridine-Hoechst staining procedure was applied (Gomez-Chavarin et al., 1987; Albertini et al., 1988). This method is a quite simple and rapid assay that detects 6-thioguanine-resistant T lymphocytes (TG<sup>r</sup> T lymphocytes) in human peripheral blood lymphocytes, and has been applied successfully for detecting HPRT<sup>-</sup> mutations in human populations (Ostrosky-Wegman et al., 1987).

## Materials and methods

### *Estimation of HPRT mutant frequency*

Venous blood samples from healthy donors and radiation-exposed individuals were collected in heparinized tubes and shipped from Goiania to The Netherlands by air. Lymphocytes were separated by Ficoll-Hypaque density centrifugation and stored at  $-70^{\circ}\text{C}$ . The culture procedure was similar to that described by Ostrosky-Wegman et al. (1987). Briefly, cryopreserved lymphocytes were grown for a 'first round' in lectin and 6-thioguanine ( $2 \times 10^{-4}$  M)-containing growth medium, followed by a 'second round' in BrdU (5  $\mu\text{M}$ )-containing medium. Cultures were terminated at 42 h, followed by a standard fixation protocol. Hoechst-stained slides were scored under a fluorescent microscope and labeling indices for thio-guanine (LI<sub>t</sub>) and control (LI<sub>c</sub>) cultures were determined. The variant frequency (VF) was determined by applying the following formulas:

LI<sub>c</sub> = Number of labeled cells per 3000 cells

$$\text{LI}_t = \frac{\text{Number of labeled cells on all TG slides}}{\text{Total number of cells on all slides}}$$

$$\text{VF} = \frac{\text{LI}_t}{\text{LI}_c}$$

### *Estimation of frequency of chromosome aberrations*

Phytohemagglutinin-stimulated peripheral blood lymphocytes from radiation victims were cultured in F-10 medium, arrested with Colcemid, treated with a hypotonic solution of 0.075 M KCl, and fixed in acetic acid-methanol. Metaphase spreads were made by the standard protocol. One set of slides was stained with Giemsa (Merck) for

conventional chromosome aberration study, while the other slides were processed for in situ hybridization study. For chromosome 1, the puc1.77 probe containing centromeric repetitive DNA was used. For the other chromosomes, whole libraries were used. Chromosome libraries were purchased from American Type Culture Collection (ATCC). Phage libraries were amplified by infecting *E. coli* LE 392 cells. DNA was isolated, purified by ammonium sulfate precipitation (Ziai et al., 1988) and biotinylated by nick translation. Under standard conditions, 10.0–30.0  $\mu\text{g}/\text{ml}$  of biotin-labeled DNA representing library inserts were combined with competitor DNA, ethanol-precipitated and resuspended in 50% formamide,  $2 \times \text{SSC}$ , 10% dextran sulfate and hybridized in situ with cytological preparations. After hybridization the slides were washed in 50% formamide/ $2 \times \text{SSC}$ , pH 7.0 ( $3 \times 5$  min, at  $44^{\circ}\text{C}$ ), followed by washes in  $2 \times \text{SSC}$  ( $5 \times 5$  min, at  $42^{\circ}\text{C}$ ) and one wash with  $4 \times \text{SSC}/\text{Tween } 20$ , pH 7.0. Thereafter, the slides were incubated with 5% natural non-fat dry milk (Lucerne Ltd., U.S.A.) for 15 min in a moist chamber at room temperature. Detection of the biotinylated probe was achieved using fluorescein-labeled avidin. All detection reagents were incubated with 5  $\mu\text{g}/\text{ml}$  fluorescein isothiocyanate (FITC)-conjugated avidin DCS (Vector Laboratories) for 20 min at room temperature, followed by 2 washes. Amplification of the signals was done by reincubation with 5  $\mu\text{g}/\text{ml}$  biotin-conjugated goat anti-avidin D antibodies (Vector Laboratories) for 20 min at room temperature followed by another incubation with Avidin D and FITC for 20 min at room temperature. The signals were amplified twice. Slides were dehydrated, dried and mounted in 20 mM Tris-HCl (pH 8.0), 90% glycerol containing 2% of antifade 1,4-diazabicyclo-[2,2,2]-octane (DABCO). Preparations were examined under a Zeiss microscope equipped with DAPI and FITC epifluorescence optics. Metaphases and interphases were scored for the presence of translocations/deletions and numerical aberrations respectively.

## Results

### *Estimation of HPRT mutant frequency*

The vFs found in 5 healthy individuals are presented in Table 1, and these range from 4.2  $\times$

TABLE 1  
HPRT<sup>-</sup> MUTANT FREQUENCY IN HEALTHY DONORS

Proband	Cells recovered	Control	Labelling index TG ( $2 \times 10^{-4}$ M)	Variant frequency
1	$3.5 \times 10^6$	0.120	$5.7 \times 10^{-7}$	$4.20 \times 10^{-6}$
2	$6.9 \times 10^6$	0.168	$1.17 \times 10^{-6}$	$6.94 \times 10^{-6}$
3	$4.6 \times 10^6$	0.175	$1.09 \times 10^{-6}$	$6.20 \times 10^{-6}$
4	$5.3 \times 10^6$	0.125	$7.57 \times 10^{-6}$	$6.00 \times 10^{-6}$
5	$4.5 \times 10^6$	0.158	$1.33 \times 10^{-6}$	$8.40 \times 10^{-6}$

TABLE 2  
HPRT<sup>-</sup> MUTANT FREQUENCY IN RADIATION-EXPOSED INDIVIDUALS

Pro-band	Estimated dose (Gy)	Cells recovered	Control	Labelling index TG ( $2 \times 10^{-4}$ M)	Variant frequency
Seven months after the accident:					
2	6.2	$9.5 \times 10^5$	0.011	$4.2 \times 10^{-6}$	$3.8 \times 10^{-4}$
3	4.4	$7.6 \times 10^5$	0.113	$2.6 \times 10^{-5}$	$2.3 \times 10^{-4}$
9	1.3	$1.2 \times 10^6$	0.024	$8.6 \times 10^{-7}$	$3.6 \times 10^{-5}$
Fifteen months after the accident:					
5	3.0	$1.6 \times 10^5$	0.050	$6.3 \times 10^{-6}$	$1.3 \times 10^{-4}$

$10^{-6}$  to  $8.4 \times 10^{-6}$ . In probands 2, 3, 4 and 5 mutant frequency was also determined by cloning assay (data not shown). Rates obtained by BrdU labeling were comparable to the mutation frequency obtained by clonal assay. Our findings are in agreement with previously reported results based on autoradiography for the detection of variants and clonal assay, where similar frequencies were found in a given sample (Albertini et al., 1988).

Table 2 summarizes vFs observed in peripheral blood lymphocytes of radiation-exposed individuals. The assay was carried out several months after the accident. Studies on all highly exposed individuals could not be carried out because of the poor viability of the lymphocytes. Seven months after the accident LIC ranged from 0.011 to 0.113, which is relatively low compared to that found in healthy donors (see Table 1). It suggests a delayed cell-cycle progression in the lymphocytes of exposed individuals. Variant frequencies were quite high in exposed individuals compared to normals. In probands 2, 3 and 9, with estimated doses of

TABLE 3  
DICENTRIC FREQUENCIES IN RADIATION-EXPOSED INDIVIDUALS

Proband	Estimated dose (Gy) <sup>a</sup>	Dicentrics + rings/cell	
		Immediately after accident	Fifteen months after accident
1	7.0	1.253	0.26
2	6.2	1.030	0.18
3	4.4	0.824	0.06
4	4.3	0.540	0.14
5	3.0	0.270	0.04
6	2.9	0.262	0.08
7	2.7	0.228	0.04
8	2.1	0.160	0.04
9	1.3	0.070	0.04
10	1.1	0.060	ND
11	1.0	0.053	0.02
12	1.0	0.050	0.02
13	0.3	0.02	0.02
14	0.2	0.005	ND

ND, not determined.

<sup>a</sup> Data from Ramalho et al. (1988).

6.20, 4.50 and 1.30 Gy, vFs were  $3.8 \times 10^{-4}$ ,  $2.3 \times 10^{-4}$  and  $3.6 \times 10^{-5}$ , respectively. Fifteen months later, in proband 5 (estimated dose 3.00 Gy) the vF was  $1.3 \times 10^{-4}$ . From the above data one can conclude that there is an elevated vF in the

TABLE 4  
FREQUENCIES OF HYPERPLOID LYMPHOCYTES AS DETECTED BY A CHROMOSOME 1-SPECIFIC PROBE (PUC1) IN RADIATION ACCIDENT VICTIMS

Proband	Estimated dose (Gy)	Cells scored	Hyperploid cells	Aneuploid cells (%)
1	7.0	894	10	1.12
2	6.2	761	4	0.52
3	4.4	1000	0	0
4	4.3	1000	7	0.7
5	3.0	846	3	0.35
6	2.9	882	3	0.34
7	2.7	ND	—	—
8	2.1	593	1	0.17
9	1.3	1000	0	0
10	1.1	ND	—	—
11	1.0	944	3	0.32
12	1.0	470	2	0
13	0.3	1000	0	0
14	0.2	1000	2	0.20

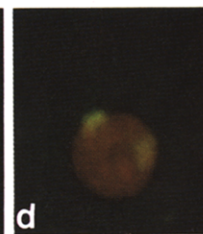
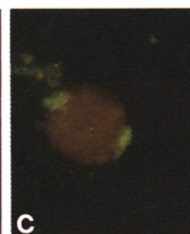
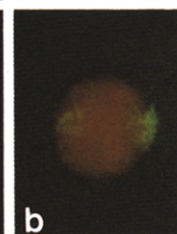
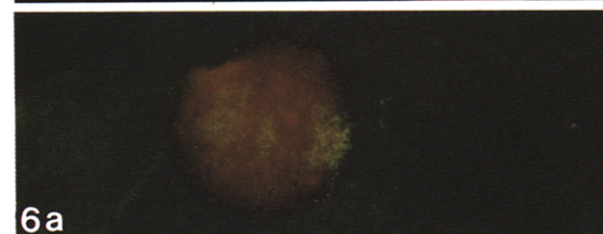
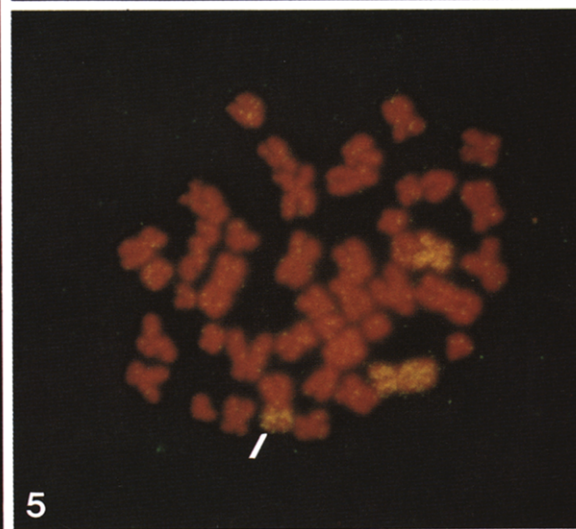
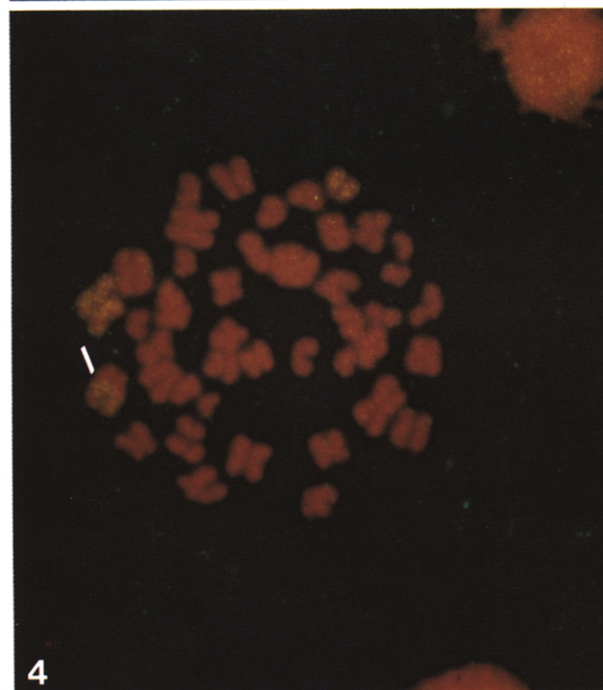
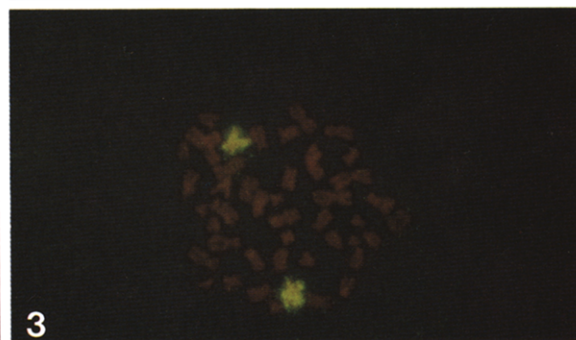
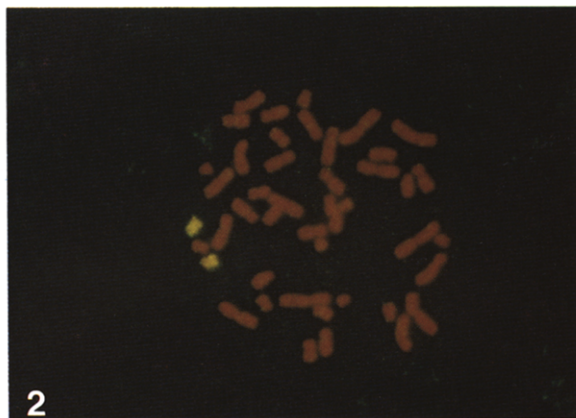
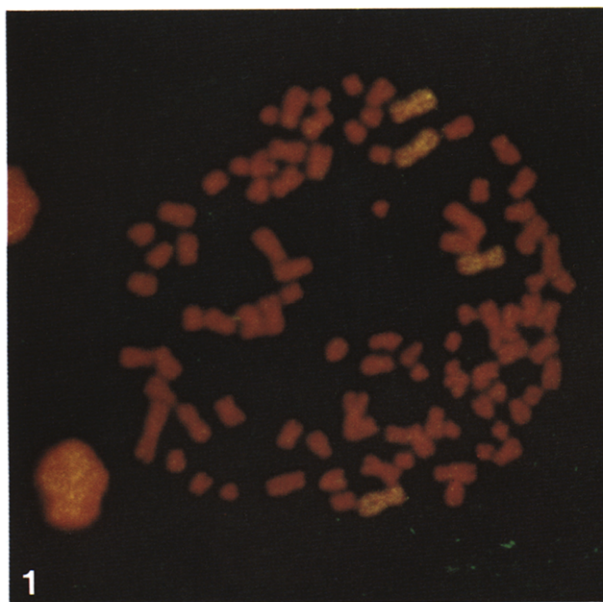


TABLE 5

DOMAINS OF CHROMOSOME 2 IN LYMPHOCYTE NUCLEI FROM RADIATION-EXPOSED INDIVIDUALS

Proband	Estimated dose (Gy)	Number of cells with signals			
		1	2	2+1 (s)	2+2 (s)
1	7.0	38	61	1	—
2	6.2	33	64	3	—
3	4.4	49	50	1	—
4	4.3	44	54	1.5	0.5
5	3.0	35	65	—	—
6	2.9	35	63	2	—
7	2.7	38	62	—	—
8	2.1	30	69	1	—
10	1.1	37	62	1	—
11	1.0	36	63	1	—
12	1.0	29	70	1	—
13	0.3	29	70	1	—
14	0.2	58	40	1	—

TABLE 6

DOMAINS OF CHROMOSOME 8 IN LYMPHOCYTE NUCLEI FROM RADIATION-EXPOSED INDIVIDUALS

Proband	Estimated dose (Gy)	% of cells with signals				
		1	2	2+1 (e)	2+1 (s)	2+2 (s)
1	7.0	—	—	—	—	—
2	6.2	32.5	67	—	0.5	—
3	4.4	35	64	0.5	0.5	—
4	4.3	22	75.6	0.4	1.6	—
5	3.0	35.3	63.2	0.3	0.9	0.3
6	2.9	32	67.6	0.3	0.3	—
7	2.7	41	59	—	—	—
8	2.1	—	—	—	—	—
9	1.3	45.8	53	0.25	0.7	0.25
10	1.1	43	55.5	0.3	0.9	0.3
11	1.0	51	47.5	0.5	1.0	—
12	1.0	42	57	—	1.0	—
13	0.3	44.5	54.2	0.3	1.0	—
14	0.2	33	66	—	0.5	0.5

lymphocytes of exposed persons, and that it persists for several months following the exposure.

#### *Frequency of chromosome aberrations*

Table 3 gives information regarding the frequency of dicentric observed in metaphase from Giemsa-stained slides. Immediately after the radiation accident (Ramalho et al., 1988), the dicentric/cell values in probands 1, 2 and 3 were 1.253, 1.030 and 0.824 and the frequencies decreased to 0.26, 0.18 and 0.06, respectively, 15 months after the accident. Depending upon the radiation dose a proportional decrease in relative frequency of dicentric was observed in all the probands. These data suggest that cells with dicentric are eliminated from the circulating system with time.

#### *Assessment of chromosome domains in interphase nuclei by in situ hybridization*

In situ hybridization with chromosome-specific libraries clearly reveals specific domains in the

interphase nuclei (Fig. 6a–d). 100–1000 interphase cells with signals for chromosomes 1, 2, 8 and 19 were scored and classified according to the number of signals. Table 4 summarizes the data on the number of lymphocytes from the radiation victims containing more than 2 copies of chromosome 1. Several individuals had a high frequency of hyperploidy for chromosome 1 though there was no dose dependence in their frequencies. In some probands (Tables 5 and 6) we found cells with 3–4 signals, some of these extra domains had the size and fluorescence intensity that denote the presence of an aneuploid cell having an extra copy/copies of that particular chromosome. Some domains were appreciably smaller in size with less intense fluorescence, indicating a deletion or a translocation involving that chromosome (Fig. 6a). Though the chromosome domains were accurately assessed in the majority of nuclei, not all nuclei presented a reliable index of chromosome constitution, since some nuclei

Figs. 1–6. Photographs showing fluorescent in situ hybridization of biotin-labeled library probes in metaphase and interphase lymphocytes. (1) Tetraploid cells with 4 copies of chromosome 8 are labeled. (2) Diploid cell with 2 chromosomes 19 labeled. (3) Diploid cell with 2 chromosomes 8 labeled. (4 and 5) Diploid cells involving translocations of chromosome 2 with break points at 2p1 (Fig. 4) and 2q2 (Fig. 5) from proband 2. (6a) Interphase cell showing 2 equal and one comparatively small domain indicating a break or translocation in chromosome 2. (6b) Interphase nucleus showing 2 unequal domains, due to a deletion in 1 copy of chromosome 2. (6c and 6d) Interphase nuclei showing 2 equal domains of chromosome 2.

TABLE 7

DOMAINS OF CHROMOSOME 9 IN LYMPHOCYTE NUCLEI FROM RADIATION-EXPOSED INDIVIDUALS

Proband	Estimated dose (Gy)	% of cells with signals			
		1	2	2 + 1 (e)	2 + 1 (s)
5	3.0	57	43	—	—
6	2.9	50	50	—	—
7	2.7	36	62	—	—
10	1.1	50	49	—	1
14	0.2	25	72	1.5	1.5

revealed only 1 domain, a few showed no signal at all and sometimes 2 domains were not clearly separable. These may be due to sub-optimal hybridization. Thus, only addition of domains, indicating hyperdiploidy, was considered in assessing aneuploid events. The frequencies were for chromosome 1, 0–12%, for chromosome 2, 0–3%, and for chromosome 8, 0–1.6% among the exposed individuals. For chromosome 19, however, the frequency was low (Table 7).

#### *Evaluation of chromosome aberrations in metaphases*

Following in situ hybridization with chromosome-specific library probes, individual chromosomes could be easily discerned (Figs. 1–3) as well as translocations involving these chro-

mosomes (Figs. 4 and 5). Table 7 presents the frequencies of translocations and deletions involving chromosome 2 in exposed individuals. In proband 2 we found 8.33% cells with deletions and 6.82% cells with translocations. In probands 3 and 4 (estimated doses 4.30 and 4.40 Gy, respectively), 5 and 3% of cells with deletions and 1 and 2% of cells with translocations respectively were detected. In proband 2, break points for the translocations and deletions (Figs. 4 and 5) occurred consistently close to or within band 2p1 (78%) and at or near to band 2q2 (22%). The frequencies of deletions and translocations in chromosome 8 are presented in Table 8. In proband 2, for whom the estimated dose was 6.2 Gy, the frequencies of deletion and translocation were 3% and 2% respectively. In proband 4 (estimated dose 4.3 Gy), we found 5% deletions and 1% translocations. In proband 12, we did not find a single metaphase with a deletion or a translocation.

#### Discussion

In September 1987, a cesium-137 radioactive source was broken open and dispersed in Goiania (Brazil), leading to considerable radiation exposure for several individuals. The initial estimation of absorbed doses was made using cyto-

TABLE 8

TRANSLOCATION/DELETION FREQUENCIES USING HUMAN CHROMOSOME 2 LIBRARY IN RADIATION ACCIDENT VICTIMS

Proband	Estimated dose (Gy)	Cells scored	Cells with deletion (%)	Cells with translocation (%)
1	7.0	43	2.30	0.00
2	6.2	132	8.33	6.82
3	4.4	100	3.00	2.00
4	4.3	100	5.00	1.00
5	3.0	132	0.00	0.00
6	2.9	63	1.59	0.00
7	2.7	39	0.00	2.66
8	2.1	61	1.60	0.00
9	1.3	125	0.00	0.00
10	1.1	115	0.87	1.74
11	1.0	—	—	—
12	1.0	64	3.12	0.00
13	0.3	100	2.00	1.00
14	0.2	100	1.00	—

TABLE 9

TRANSLOCATION/DELETION FREQUENCIES USING HUMAN CHROMOSOME 8 LIBRARY IN RADIATION ACCIDENT VICTIMS

Proband	Estimated dose (Gy)	Cells scored	Cells with deletion (%)	Cells with translocation (%)
1	7.0	—	—	—
2	6.2	100	3	2
3	4.4	66	1.6	—
4	4.3	82	5	1
5	3.0	120	3	—
6	2.9	100	2	—
7	2.7	12	—	—
8	2.5	—	—	—
9	1.3	100	1	—
10	1.1	100	2	1
11	1.0	64	1.6	—
12	1.0	100	—	—
13	0.3	100	4	—
14	0.2	100	4	—

genetic analysis of stimulated blood lymphocytes of the victims (Ramalho et al., 1988). Since then, there have been follow-up studies, using blood periodically sampled from some of these highly exposed individuals. The frequency of dicentric was found to decrease with time in most cases in whom the exposure was mainly external; in 2 individuals who had internal contamination, the frequency of dicentrics increased up to 100 days and then declined (Ramalho et al., 1990).

We have performed cytogenetic analyses in the blood lymphocytes of the victims to study the frequencies of dicentrics (15 months after the accident), the frequencies of translocations, deletions and aneuploid events using chromosome-specific probes. The dicentric frequencies decreased (Table 3) in comparison to initial frequencies, and are comparable to the data obtained by Ramalho et al. (1990). It is known from earlier follow-up studies of radiation-exposed individuals, such as Hiroshima–Nagasaki atom bomb victims, as well as after other radiation exposures, that dicentric frequencies decline with time (Buckton et al., 1978; Bauchinger et al., 1968; Awa et al., 1978). However, the frequencies of translocations determined with the conventional banding technique remain high, as clones for some stable aberrations can persist for a long time. The use of chromosome-specific probes to detect translocations is more sensitive than conventional techniques because of the accuracy and ease of detection as well as the large number of cells that can be analyzed. For example, it is assumed that radiation-induced reciprocal translocations and dicentrics in lymphocytes are formed in an equal proportion of 1:1. When translocation frequencies are detected using specific probes, they appear to be much greater than dicentric frequencies at the same dose (Lucas et al., 1989; Vyas and Natarajan, 1990).

By applying *in situ* hybridization, individual chromosomes can be directly visualized as discrete domains in interphase nuclei (Manuelidis et al., 1985; Pinkel et al., 1986; Cremer et al., 1988a,b; Litcher et al., 1988). Detection of aneuploidy and loss of chromosome fragments in interphase cells was carried out using chromosome-specific probes for chromosomes 1, 2, 8 and 19. Most of the cells examined had 1 or 2 signals. This excess of hypodiploid cells is likely to be due to technical

artifacts. Cells without a signal were also seen but they were not considered for scoring. An occurrence of 1 or 2 extra unequal domains is considered evidence for the presence of 3 or 4 chromosomes, denoting a deletion or translocation involving that chromosome (Fig. 1). One or 2 extra signals with equal fluorescent intensities suggest the presence of a trisomic or tetrasomic cell. Among the individuals studied, we did not find any relation between the absorbed radiation dose and the frequency of lymphocytes with numerical aberrations. In order to check the specificity of the signals generated in interphase nuclei, a parallel analysis was made in metaphase preparations. Frequencies of translocation and deletion were found to decrease in the order: chromosome 2, 8, 19. This suggests that though there is inter-individual variation, chromosome breakage depends mainly upon the length of the chromosome. Deletion and translocation frequencies in chromosome 2 were higher than in chromosomes 8 and 19. The elevated deletion and translocation frequencies in chromosome 2 in different individuals correlate well with the frequencies determined for chromosome 8. These findings indicate that lymphocytes of these individuals carry a translocation or deletion involving more than 1 chromosome. In proband 2, the deletion and translocation frequencies in chromosome 2 were much higher than in any other proband studied. Furthermore, we found 2 consistent break points in chromosome 2, which were confirmed by the DAPI-dystamycin banding pattern. These break points were found to be close to or within band 2p1 (Fig. 4) and 2q2. A high incidence of specific translocations could be due to the clonal origin of these cells, but this cannot be verified at present. A more precise definition of break points and of chromosomal regions involved in deletion and translocation can be achieved by using either single-copy probes or region-specific repetitive probes. Since these break points are found to be associated with several hematological disorders and as translocation plays a crucial role in tumor development (Mittelman, 1988), these preliminary findings may provide some clues for the early detection of malignancy.

The frequency of mutations in the HPRT locus in human lymphocytes has been shown to be a good indicator of exposure to genotoxic agents



including ionizing radiations (Messing and Bradley, 1983; Sanderson et al., 1984; Vijayalaxmi and Evans, 1984). The detection of these mutations by labeling ( $^3\text{H}$ -TdR or BrdU) seems to be as efficient as by cloning (Albertini et al., 1988; our unpublished results). In the present study, we detected a very high frequency of HPRT mutants and the induction was dose-dependent (Table 2). Proband 2 received partial body exposure, hence the frequency is lower than expected. Since we determined the frequencies of mutants with the labeling method, we cannot say whether each mutant cell arose as an independent mutant or not; some of them could be of clonal origin. Further studies involving sequencing of mutants will answer this question. In a parallel investigation, we have found increased frequencies of hemoglobin mutants in these individuals (Bernini et al., unpublished results).

In conclusion, the follow-up study of the radiation victims of Goiania shows that (a) dicentric frequencies in lymphocytes decrease with time; (b) based on the detection method using DNA probes, translocations and deletions involving the tested chromosomes 2, 8 and 19 are high; (c) aneuploidy is induced in lymphocytes by radiation and they persist for a long time; and finally (d) the frequency of HPRT mutations is also increased in these individuals. All the parameters studied, namely, point mutations, chromosomal translocations and deletions as well as numerical aberrations, are known to be involved in human neoplasms. The data collected in this study as well as others of a similar nature would enable one to see whether any of these studied parameters is predictive of the future occurrence of neoplasms in these individuals.

## Acknowledgements

We are grateful to Dr. Adriana Ramalho, IRD/CNEN, Rio de Janeiro for her help in collection and transportation of blood samples. This study was partly supported by a study grant to A.T.N. from the CEC Radiation Protection Programme.

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