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ABSTRACT

Purpose: The purpose of this study was to examine a new approach to retrospective biological dosimetry, by using a long-term animal model to determine the stability of translocation frequency after *in vivo* irradiation. While the frequency of dicentrics is known to decrease over time, the persistence of more stable chromosomal aberrations such as translocations could be useful if their stability were definitively proved.

Materials and Methods: Four monkeys (*Macaca fascicularis*) were exposed to two different doses of ionising radiation: 2 Gy whole body irradiation for two and 4 Gy for two others. Blood samples were obtained at various times after irradiation. Both total and two-way translocations were detected by fluorescence *in situ* hybridisation. Translocations were scored in stable cells, that is, those without dicentrics, rings or fragments. The course of translocation frequency was analysed at four time-points: one hour (H1), 2 months (M2), 10 months (M10) and 31 months (M31) after irradiation.

Results: We observed two separate trends in translocation frequency: total translocation frequency decreased slightly in animals irradiated with a dose of 2 Gy, while two-way translocation frequency was relatively stable in all irradiated animals.

Conclusions: We confirmed the long-term stability of translocations and found that it seems to depend on the type of the translocation recorded. Overall translocations were stable for up to 31 months regardless of dose, but two-way translocations were more stable than those that were non-reciprocal, especially in stable cells.

INTRODUCTION

The standard procedure for biological estimation of radiation doses in cases of accidental overexposure consists in scoring unstable chromosomal aberrations (dicentrics and/or centric rings) in peripheral blood lymphocytes. This method is currently considered to be both the most specific and most sensitive for short-term biological dosimetry (Bender et al. 1988, Voisin et al. 2001, Duran et al. 2002). Several months after exposure, however, this conventional cytogenetic method is no longer the most effective way to assess radiation doses, since the frequency of cells carrying these chromosomal aberrations decreases with time after exposure (Sorokine-Durm et al. 2000). Dose assessment may therefore be difficult when the time elapsed between exposure and analysis either exceeds one year or is unknown. Retrospective dose estimation based on the frequency of stable aberrations, such as translocations, may therefore be preferable, as suggested by Sorokine et al. (1997). The fluorescence *in situ* hybridisation (FISH) technique makes it easy to detect these aberrations. Determining its accuracy for retrospective biological dosimetry, however, requires verification of the persistence of translocation yields in lymphocytes for several years after irradiation.

The subject of the persistence of translocations has elicited much controversy in the literature. On one hand, some publications showed stability of translocation frequencies as follows. Studies of both Hiroshima A-bomb survivors (Lucas et al. 1992, Lucas et al. 1996) and whole-body irradiated rhesus monkeys (Lucas et al. 1996) show high levels of translocation stability. In both analyses, translocation frequencies were not found to change over a period of up to 30 years after exposure, but the initial translocation frequencies were not measured. For the Hiroshima A-bomb survivor study, translocation dose estimates from survivors' lymphocytes obtained 30 years after the 1945 bombing were compared with estimated doses from *in vitro* dosimetry curves. For rhesus monkeys exposed to whole-body irradiation, translocation frequencies were compared with doses delivered 28 years ago, measured by physical dosimetry. Translocation frequency also remained stable in an individual exposed to tritium according to a comparison of initial dicentric frequency with translocation frequency, measured eleven years later (Lucas et al. 1993). A study of several Estonian radiation-accident victims also found that translocation yields for three subjects remained relatively constant for more than two years (Lindholm

et al. 1998).

Results differed, on the other hand, for another victim of the same radiation accident, a 13-year-old boy exposed to protracted low dose-rate, whole-body and short-time partial-body irradiation. The frequency of radiation-induced translocations in his peripheral blood lymphocytes decreased significantly (Bauchinger et al. 2001, Lindholm et al. 2004). The decrease in this case may be due to either the heterogeneous irradiation or the high received dose, or both (Guerrero-Carbajal et al. 1998). For the Goiania accident, translocation frequencies, analysed one year after exposure, were compared with the initial dicentric frequencies. The authors observed translocation yields two to three times lower than the initial dicentric yields (Darroudi 2000). Some animal studies also report decreases in translocation frequency over time (Spruill et al. 2000). The principal advantage of animal studies is that translocations can be scored just after exposure and exposure condition can be controlled. In mice exposed to a 2-Gy dose of X-rays, the translocation yield decreased for up to 21 months after irradiation (Spruill et al. 2000) and in mice exposed to ^{137}Cs γ–rays at doses ranging from 0 to 4 Gy, these yields decreased for up to 7.5 months (Hande et al. 1998).

Samples obtained shortly after irradiation or even a few months later show higher translocation frequencies than those measured one year or more after irradiation. That is, this stability depends on the conditions of irradiation (whole- or partial-body irradiation) and the period between radiation exposure and the initial biological dose estimation (Darroudi 2000).

It is thus difficult to draw clear-cut conclusions about the persistence of chromosomal translocations especially in humans who are accidentally irradiated, often in complex configurations. To understand the course of translocation frequency after exposure to ionising radiation, we studied lymphocytes from monkeys acutely irradiated at doses of 2 and 4 Gy for nearly three years after exposure. This paper reports the translocation and dicentric frequencies we observed over time and the conclusions we have drawn.

MATERIALS AND METHODS

Animals and Irradiation procedure

Four young male macaque monkeys (*Macaca fascicularis*) weighing an average of 5.0 ± 0.5 kg were used. We chose this species because of its similarity to humans in terms of both cytogenetic profile and biological response to radiation. The animals were housed in individual stainless steel cages with a constant room temperature of 23 °C and normal daylight rhythm. They received sterile water, biscuits *ad libitum* and peeled fruits. All experimental procedures were approved by the IRSN (Institute for Radiological Protection and Nuclear Safety) Animal Care Committee and complied with French regulations for animal experimentation (Ministry of Agriculture Decree no. 87-848, 19 October 1987, amended on 29 May 2001). The four monkeys used in this study were exposed to "whole-body" irradiation with γ rays, at a dose-rate of 0.1Gy.min^{-1} (^{60}Co; ICO 4000, Fontenay aux Roses, France) for doses of 2 Gy (animals #1 and #2) and 4 Gy (animals #3 and #4). Animals were kept and the irradiation performed in close proximity of the laboratory. The animals did not receive any other treatment such as bone marrow transplantation (Bertho et al. 2001).

Culture procedure

Blood aliquots were sampled in heparinized tubes at different times after exposure: 1 hour (H1), 2 months (M2), 10 months (M10), 17.5 months (M17.5) and 31 months (M31). Blood was also sampled five days before irradiation and at 24 hours after irradiation, for lymphocyte counts only. Lymphocytes were counted by the ADVIA 120 hematology system (Bayer Diagnostics, Puteaux, France). The lymphocyte culture procedure was previously described in Sorokine-Durm et al. (2000). Briefly 1 ml whole-blood sample was cultured at 37 °C and 5% CO_2 in 10 ml of RPMI (Roswell Park Memorial Institute Medium) 1640 (Life Technologies, Cergy Pontoise, France) supplemented with 10-20% (depending on lymphocyte count) foetal calf serum (FCS) and 5% phytohemagglutinin-M, in the presence of 2 mM L-glutamine, 1 mM pyruvate, 10 mM Hepes buffer and 1% penicillin-streptomycin (all from Life Technologies, Cergy Pontoise, France). In some experiments, culture conditions were

modified by varying culture duration, percentage of FCS and inclusion of interleukin 2 (IL-2, 1ng.ml^{-1}, Tebu, Le Perray en Yvelines, France), to improve T cell activation. To prevent scoring of dicentrics among second-division cells, 1% of bromodeoxyuridine (BrdU, 500µg.ml^{-1}, Sigma-Aldrich, St Quentin Fallavier, France) was added to the culture medium. A mitotic inhibitor (Colcemide, Life Technologies, Cergy Pontoise, France) was added 2 or 4 hours before the end of the culture to block cells in the metaphase stage. Cells were then harvested, treated by hypotonic shock (0.075 M KCl, Sigma-Aldrich, St Quentin Fallavier, France), and fixed three times in methanol/acetic acid (3/1 v/v; VWR, Fontenay-sous-bois, France), followed each time by a centrifugation step. Metaphases were spread out on glass slides for analysis. The fluorescence plus Giemsa (FPG) staining technique was used for conventional dicentric scoring (Sorokine-Durm et al. 2000). Slides for FISH painting were stored at –20 °C until use.

Chromosome Painting

The FISH-painting technique used for monkey chromosomes has previously been described in detail (Sorokine-Durm et al. 2000). Briefly, after slides were treated with RNAse (RNAse A, 1 mg.ml^{-1}, Roche Diagnostics, Meylan, France), they were processed by pepsine digestion (1mg.ml^{-1}, Roche Diagnostics, Meylan, France). A formaldehyde solution (500 ml, Sigma-Aldrich, St Quentin Fallavier, France) was used in the post-fixation phase, and the slides were dehydrated in ethanol series. The slides were then incubated in the presence of a hybridisation mixture containing the chromosome-specific DNA probes: first, for 2 minutes at 70° C using the HYBrite system (Abbott, Rungis, France) to denature both chromosomes and probes simultaneously, and then for 16 hours at 37° C to allow hybridisation of probes. Lucas et al. (1993) and Wienberg et al. (1992) demonstrated the cross-reactivity of human probes for painting *Macaca sp.* chromosomes (Wienberg et al. 1992). We used probes for human chromosomes 1 and 4 (Qbiogen, Illkirch, France) to paint the same chromosomes in *M. fascicularis* with no cross hybridisation to other chromosomes (Blakey et al. 1993), and probes for human chromosome 13 (Qbiogen, Illkirch, France) to paint monkey chromosome 16 (Ward et al. 1994). The probe for human chromosome 1 was coupled with fluorescein isothiocyanate dye (FITC, QBiogen, Illkirch, France) to paint the chromosome in green, the probe for chromosome 4 coupled

with Texas Red dye (TR, QBiogen, Illkirch, France) to paint it in red, and the probe for chromosome 13 coupled with a combination of FITC and TR to paint it in orange. All probes were mixed together. After overnight hybridisation, slides were washed once in 0.5x saline sodium citrate solution (VWR, Fontenay-sous-Bois, France) at 72 °C for 2 minutes and then once in 1x phosphate buffer detergent (Qbiogen, Illkirch, France) for 1 minute at room temperature. Chromosomal DNA was counterstained with 4.6-diamidino-2-phenylindole-2-hydrochloride (DAPI, Qbiogen, Illkirch, France) in an antifading solution that contained para-phenylene-diamine-dihydrochloride (PPD, Sigma-Aldrich, St Quentin Fallavier, France). FISH slides were analysed with a fluorescence microscope (Nikon, Micromécanique, Evry, France) equipped with filter blocks for simultaneous observation of up to three different fluorescence signals. A simple band-pass DAPI filter was used to check chromosome shapes (Sorokine-Durm et al. 2000).

Aberration Scoring

We scored mainly cells in first division. To be accepted for scoring, metaphases in first division had to be well spread out and contain both the full complement of chromosomes and, as far as could be ascertained, all the material of the six painted chromosomes (Sorokine-Durm et al. 1997, Hone et al. 2005).

Only metaphases with complete painted patterns were considered and analysed for translocation scoring. Between 500 and 1,500 metaphases were scored per sample. A bicolour chromosome with a single centromere in the painted part was classified as a two-way translocation (TwT) when its reciprocal bicolour counterpart was present. One-way translocations contained a bicolour chromosome with a single centromere in the painted part accompanied by a painted fragment. Total translocations (Ttot) were defined as the sum of TwT and one-way translocations. Insofar as possible, only translocations in painted chromosomes in cells that had no dicentrics, fragments or centric rings in the painted and the counterstained chromosomes were scored. Such cells can pass throughout cellular divisions, and be considered more stable over time. Complex exchanges were defined as those containing more than three breaks in at least two chromosomes in painted material. We decided not to score translocations in cells containing all complex exchanges.

We scored all types of dicentrics and rings, with and without fragments. About 30 to 500 metaphases were scored per time points for each monkey. After treatment with Hoechst 33258 (Sigma-Aldrich, St Quentin Fallavier, France) and ultraviolet light to distinguish the first from the second cell divisions, slides were stained following the standard protocol for fluorescence plus Giemsa (FPG) (Sorokine-Durm et al. 2000) to score them.

Statistic Analysis

A Z-test was used to compare the yields of chromosomal aberrations obtained under different culture conditions. We used the multi-colour equation published by Lucas et al. (1992) to obtain the genomic equivalent translocation frequency: $F_G = 3.18\ F_P$ where F_G is the translocation frequency for the whole genome and F_P the translocation frequency for painted chromosomes (Sorokine-Durm et al. 2000). A chi-square test was also used to compare translocation frequencies with reference values, based on a Poisson statistic with a confidence interval of 95% (CETAMA 1998). If the difference between the two frequencies was not significant (ns: not significant), frequencies were considered stable between the 2 time points. On the contrary, if the difference was significant ($p<0.05$), we concluded that frequency was not stable during the period in question. Chi-square tests were performed for all comparisons.

RESULTS

Influence of culture conditions

Translocation frequency did not vary significantly according to a variety of culture conditions that we tested: the presence or absence of IL2, whether FCS accounted for 10% or 20% of the medium, and whether the culture lasted two or three days. These results are consistent with previously published results (Small et al. 1985, Tatsumi et al. 1986).

Follow-up of lymphocyte counts after irradiation

Five days before irradiation, all monkeys had a normal lymphocyte counts, between 3×10^9 and 5×10^9 lymphocytes.l^{-1} of blood (Figure 1).

[Insert Figure 1 about here]

One hour after irradiation, lymphocyte counts had not changed significantly, except in animal #2, irradiated with a 2-Gy dose, whose lymphocyte count rose to 8.52 x 10^9 lymphocytes.l^{-1} (Figure 1A). Twenty-four hours after irradiation, lymphocyte counts had fallen in all animals approximately 70% from the pre-irradiation values. Subsequent lymphocyte recovery was also observed, but its timing differed according to dose. For animals with 2-Gy doses, the lymphocyte count began to increase at M2 after irradiation. For animal #1, it returned to its pre-irradiation level at M10, but for animal #2 it never did it. The lymphocyte count for animal #2 remained at subnormal values. The lymphocyte count remained stable up to 31 months after exposure in both animals, from M10 for #1 and from M2 for #2 (Figure 1A). For animals #3 and #4 irradiated at 4 Gy, the lymphocyte count returned to normal range at M2, although for the former, substantial variations occurred from M10 through M31 (Figure 1B) while for the latter, it remained normal throughout the rest of the study. After lymphocyte recovery, health status was normal for all animals during the follow-up period: this observation indicates that the variations in lymphocyte counts were not linked to any specific disease.

Dicentric follow-up after irradiation

All types of dicentrics and centric rings, with and without fragments, were scored by FPG staining. Table 1 presents the data for each unstable aberration for each animal. All the visible aberrations were classified as dicentrics or centric rings, both with and without fragments involving all 46 chromosomes.

[Insert Table 1 about here]

Figure 2 summarises the unstable aberration frequencies.

[Insert Figure 2 about here]

Dicentric frequencies clearly decreased over the 31-month period following irradiation, for both dose groups (Figure 2).

By M2 more than 70% of the cells carrying unstable aberrations had disappeared in the animals with 2-Gy doses, and at M31, no dicentrics or rings were observed. The decrease for the 4-Gy irradiated animals also began by M2. More than 90% of cells with unstable aberrations had disappeared by M10 and dicentric rates at M31 were close to zero. The wide confidence interval for dicentrics at H1 is due to the inadequate number of cells obtained on these slides.

Translocation frequency variations after irradiation

Translocations were scored only in stable cells, that is, those without dicentrics, rings or acentrics. Scoring in stable cells allows these stable aberrations to be observed through several cell division cycles. We decided not to score translocations in cells with any complex exchanges, even though some of them are described stable, such as insertions.

Figure 3 depicts the course of translocation yields from irradiation through study end.

[Insert Figure 3 about here]

Figure 3A reports total translocation frequencies at the different measurement points. A dose effect was observed for all translocations, with chromosomal aberration yields at 4 Gy almost twice those at 2 Gy (Figure 3A). Chi-square tests were performed to assess stability (Table 2).

Total translocation frequency for one of the animals with a 2-Gy dose (#2) was stable from H1 to M10 but not from M10 to M31 ($p<0.05$). For the other monkey in this group (#1) it was unstable from H1 to M10 ($p<0.05$). These results show a slight decrease between H1 and M10 for both these animals, with stability only from M10 to M31 (Table 2).

[Insert Table 2 about here]

We were unable to count total translocation frequency at H1 in the animals with 4-Gy doses, because too few cells could be analysed. Chi-square tests showed stable total translocation frequencies from M2 to M31 for both animals. A slight decrease was noted at M10 for the two monkeys that were unstable from M2 through M10.

Two-way translocation yields were stable from H1 to M31 in both animals with 2-Gy doses (Figure 3B). The TwT frequencies were not significantly different from the mean value (Table 2). Overall two-way translocation frequencies appear to be stable in both animals receiving 4-Gy doses (#3 and #4), at M31 (Table 2). However, significantly reduced frequencies were observed at M10 for both animals and at M17,5 for #4. In general the chi-square values indicate that the two-way translocation frequencies were much more homogeneous than the frequencies for all translocations.

DISCUSSION

Analysis of cases of suspected overexposure several months after the fact, that is retrospective biological dosimetry, requires a biological response to ionizing radiation that is stable over the long term. Scoring "stable" chromosomal aberrations such as translocations is therefore of growing interest, but the stability of these chromosomal aberrations remains uncertain, and definitive proof is necessary to use them to assess radiation doses long after exposure. The FISH-painting technique makes it easy to distinguish translocations so that their persistence over time can be studied adequately. Using animal model in this study allowed homogeneous irradiation at a known dose together with an observation of the living conditions after irradiation. Finally it gave us the opportunity to observe both dicentric and translocation rates shortly after irradiation (H1) and at subsequent time-points up to 31 months after exposure.

The analysis of drastic influence of the translocation scoring in stable cells was widely discussed. Translocation frequency was more homogeneous in stable cells than in all cells (Lindholm et al. 2004). Although translocation yields is smaller in stable cells than in all cells, they remained more constant over time (Edwards et al. 2005).

This study also allowed us to compare the initial dicentric and translocation rate and thereby learn more about the production of these chromosomal aberrations. We found similar frequencies for both

types of radiation-induced aberrations at the initial sampling time, H1. This confirms previous reports that ionising radiation induces similar yields of dicentrics and translocations (Straume et al. 1993, Bauchinger 1998, Lindholm et al. 2002).

As expected, the frequency of dicentrics decreased from H1 to M31. Indeed, about 60% disappeared during the first two months. This early disappearance is due mainly to 70% decline in the lymphocyte counts 24 hours after irradiation of all four monkeys (Figure 2). This decline was followed by intense mitotic activity in the residual lymphoid population, which led to restoration of baseline cell counts. Some unstable chromosomal aberrations vanished during cell divisions, thus proving that these types of aberrations cannot pass through repeated cell division cycles (Voisin et al. 2000, Edwards et al. 2005).

Translocations (total and two-way) were monitored over time to study their evolution. After 2-Gy and 4-Gy exposure, total translocation frequency decreased slightly between H1 and M10. Other studies that included time-points shortly after irradiation in their analysis also report similar decreases over the first year (Spruill et al. 2000, Lindholm et al. 2004). They showed that this decrease is associated with the deletion of damaged cells carrying such unstable aberrations as dicentrics, rings and acentrics. However in our study, insofar as possible translocations in cells with dicentrics, rings or acentrics were not taken into account.

The decrease of total translocation frequency observed may be also due to the disappearance of cells carrying complex exchanges. Those cells generally described as unstable throughout cell division were excluded from translocation scoring (Edwards et al. 2005). Accordingly we scored only apparently simple translocations. Studies with the multicolour-FISH (M-FISH) technique, however, show that not all apparently simple translocations are real simple exchanges between two broken chromosomes: many of them appear to be complex exchanges (Tucker et al. 1997, Camparoto et al. 2003). Use of this technique should thus prevent the misclassification of apparently simple cell and make it possible to evaluate cell stability in more detail.

The decrease in total translocations may also be linked to the radiation-induced changes in lymphocyte counts, which dropped markedly 24 hours after exposure and recovered to initial levels by M10. This recovery necessarily entailed cell division that eliminated unstable complex aberrations and apparently

simple translocations. The early decrease may therefore have favoured the disappearance of the translocations present in complex cells.

Cell division associated with the recovery is restored once the lymphocyte count reaches its baseline level, here at M10. Thereafter peripheral blood cells are replenished by mature lymphocytes produced by irradiated stem cells after cell divisions (Lindholm et al. 2004, Edwards et al. 2005). These mature lymphocytes are obtained after several cell divisions. It can thus be assumed that all the cells presented at M10 and afterwards contain mainly stable chromosomal aberrations. This is consistent with the stability of total translocations noted from 10 to 31 months after irradiation at both doses.

We analysed translocation yields to determine if the stability profile of these two kinds of translocations (total and two-way) differs. Insofar as possible, we scored two-way translocations only in cells without dicentrics, rings or acentrics. Using these scoring criteria, we observed similar frequencies of two-way translocations throughout the study period for both doses. Two-way translocation frequencies appeared more stable in all four irradiated monkeys than total translocation frequencies. Other studies also report that two-way translocations are more stable than non-reciprocal translocations (Lindholm et al. 2004, Rodriguez et al. 2004). The preservation of genetic information and consistent chromosome shape, when two-way translocations are produced, may explain this finding. Cell division is therefore not altered. As long as no gene activation or inactivation crucial for cell survival occurs (Camparoto et al. 2003), there is no reason why cells containing two-way translocations should disappear. *In vitro* studies have confirmed the stability of two-way translocation yields from first to second generation cells, while non-reciprocal translocation yield decreases (Pala et al. 2001).

In conclusion, we confirm in this study that the analysis of two-way translocation frequency appears more useful for retrospective assessments of past radiation doses, as long as specific criteria are met: they must be scored only in cells without unstable chromosomal aberrations and complex exchanges. The use of non-human primates allowed us to demonstrate this stability for longer than 2 years, even though we studied only a few animals.

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FIGURES

Figure 1*: Lymphocyte counts over time in irradiated animals. Results consist of data from individual animals (A): closed square, animal #1; closed rhombus, animal #2; (B): closed triangle, animal #3; closed square, animal #4. Lymphocyte counts before and after homogeneous whole-body irradiation (^{60}Co; 0.1 Gy/min) are presented for 2 Gy irradiated monkeys (A): #1 ■ black line and #2 (♦) grey line. For 4 Gy irradiated monkeys (B): #3 (▲) black line, and #4 (■) grey line.*

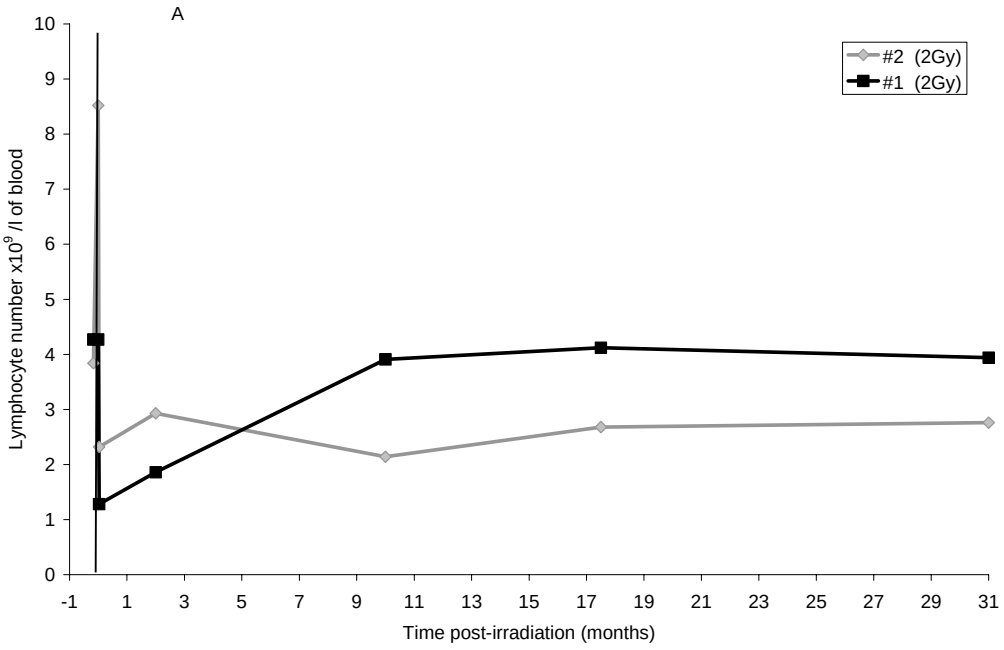
Figure 2*: Dicentric time course after irradiation at 2 Gy (black bars) and 4 Gy (white bars). Data are the mean of the two animals in each radiation dose group. Error bars represent 95% confidence interval. C represents unstable aberration frequencies before irradiation.*

Figure 3*: Genomic equivalent translocation time course after γ ray ^{60}Co irradiation (0.1 Gy/min). The course of translocations over time was analysed for total translocations (A) and for two-way translocations (B). Error bars represent 95% confidence interval.*

Table 1: *Distribution of unstable aberrations after conventional staining for each monkey at each post-irradiation measurement point.*

Table 2: *Number of translocations for each animal at each post-irradiation measurement point. Statistical analysis of stability against genomic translocation frequency at 1hour or 2 months after irradiation by chi-square test on two frequencies, comparison is performed with a 95% confidence interval.*

Figure 1


A
#2 (2Gy)
#1 (2Gy)
Lymphocyte number x10^9 /l of blood
Time post-irradiation (months)

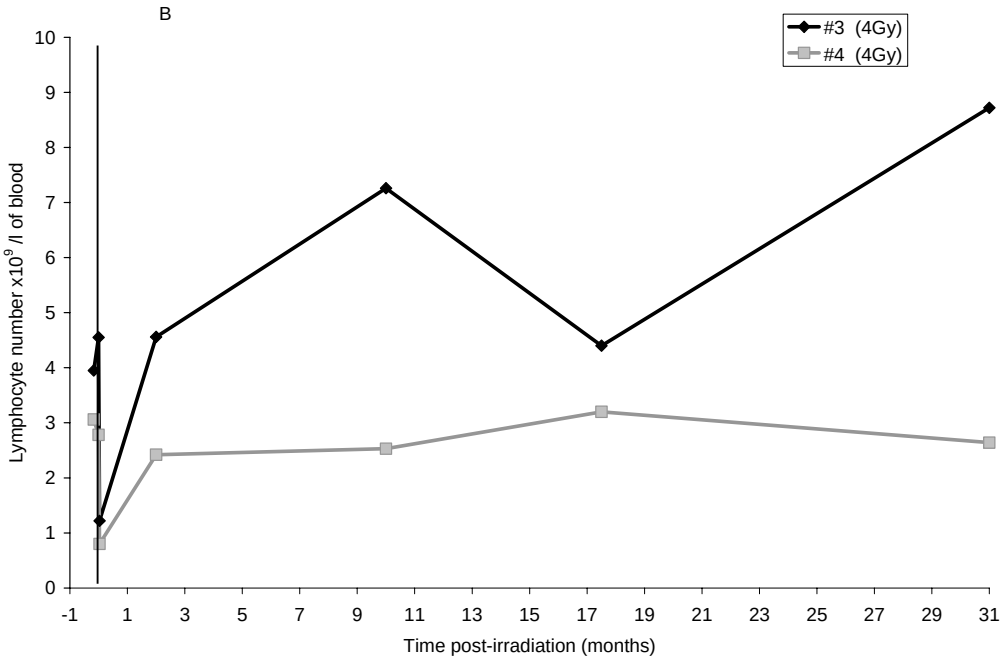

B
#3 (4Gy)
#4 (4Gy)
Lymphocyte number x10^9 /l of blood
Time post-irradiation (months)

Figure 2

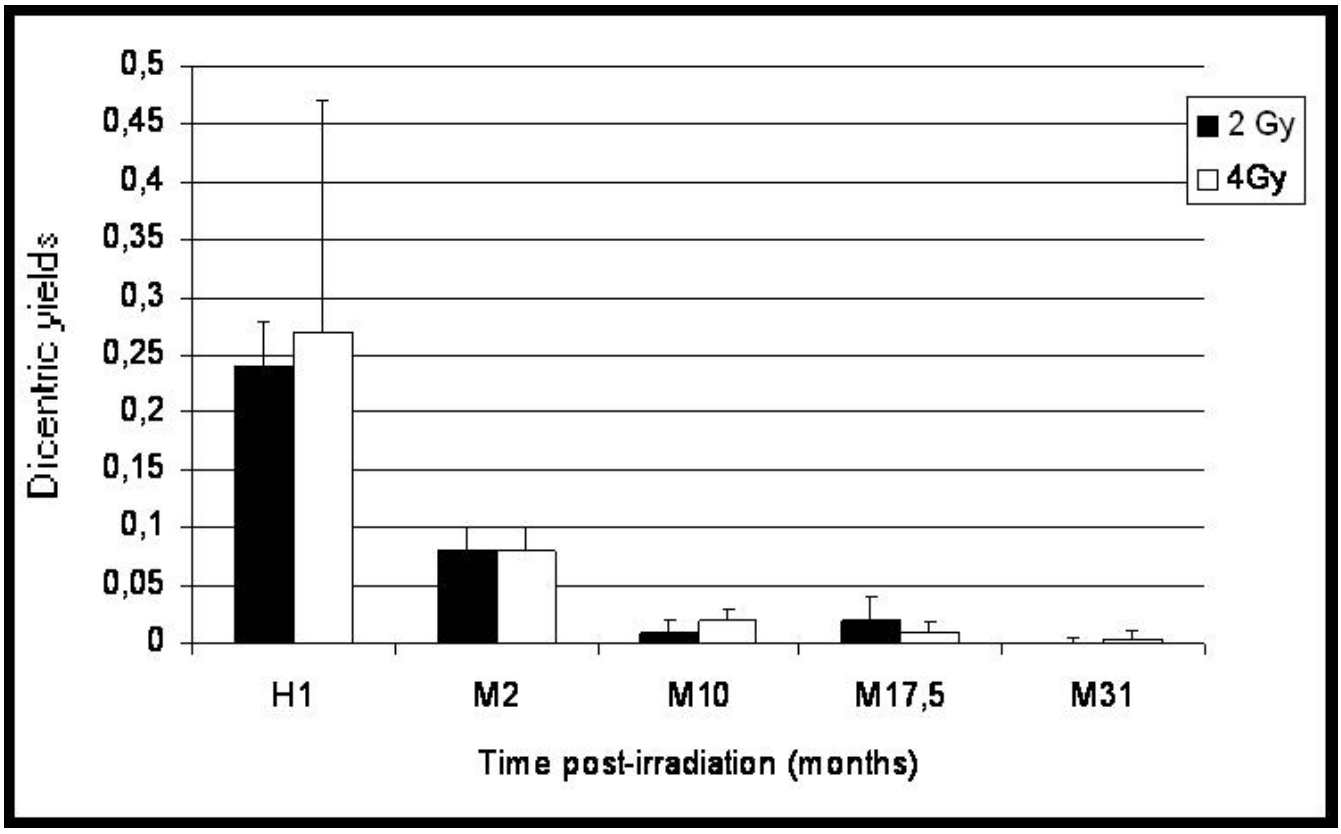
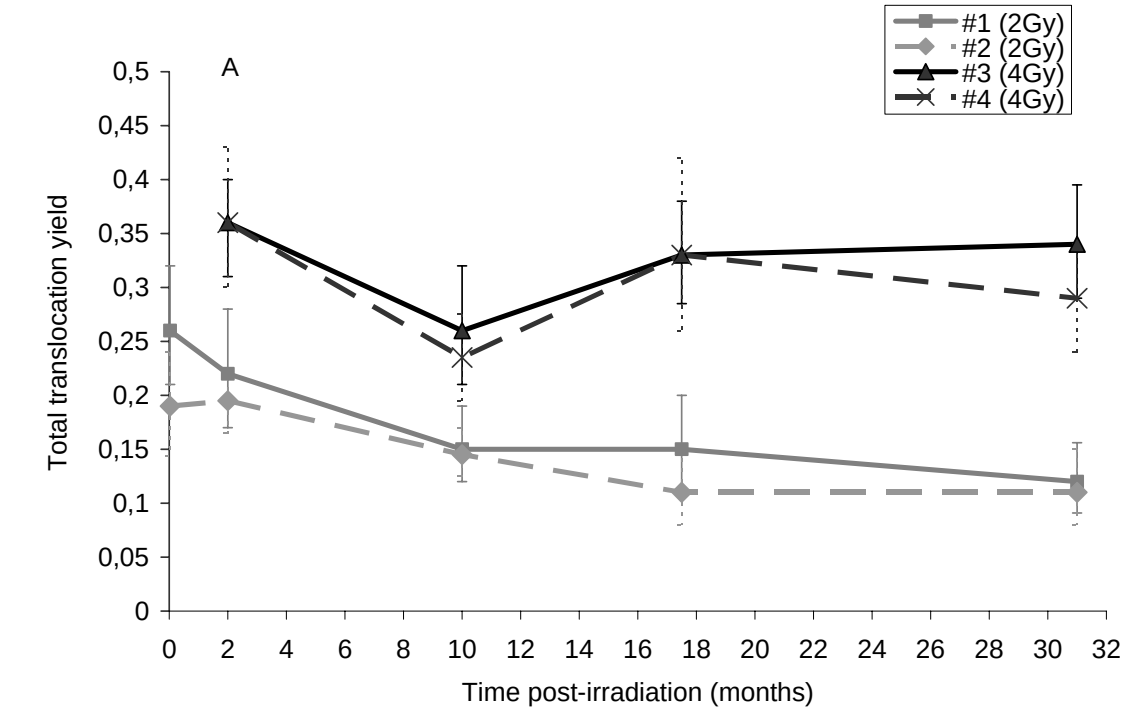

0,5
0,45
0,4
0,35
0,3
0,25
0,2
0,15
0,1
0,05
0
Dicentric yields
2 Gy
4Gy
H1
M2
M10
M17,5
M31
Time post-irradiation (months)

Figure 3


A
#1 (2Gy)
#2 (2Gy)
#3 (4Gy)
#4 (4Gy)
Total translocation yield
0,5
0,45
0,4
0,35
0,3
0,25
0,2
0,15
0,1
0,05
0
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32
Time post-irradiation (months)

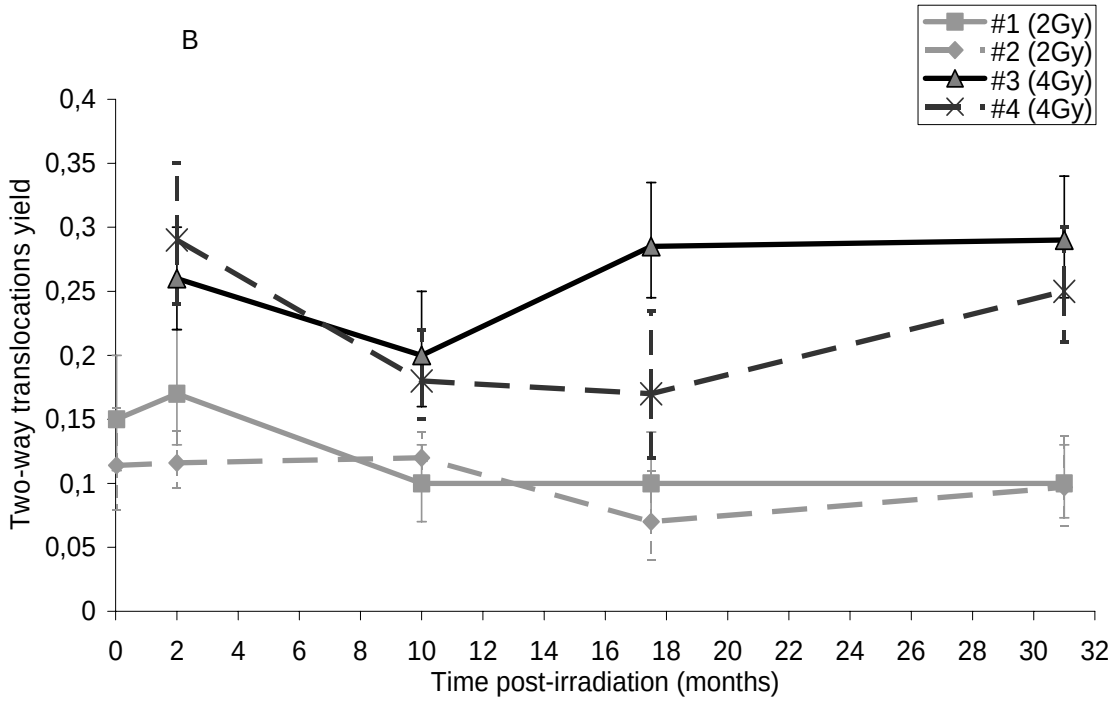

B
#1 (2Gy)
#2 (2Gy)
#3 (4Gy)
#4 (4Gy)
Two-way translocations yield
0,4
0,35
0,3
0,25
0,2
0,15
0,1
0,05
0
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32
Time post-irradiation (months)

Table 1

animal	**Dose**	**Time**	**Cells observed**	D+F	D-F	Rc+F	**Frequency**
#1	2 Gy	H1	454	90	14	8	0.25
		M2	464	30	14	0	0.095
		M10	500	1	1	0	0.004
		M17.5	200	2	1	0	0.015
		M31	514	0	0	0	0
#2	2 Gy	H1	456	100	2	3	0.23
		M2	482	18	16	0	0.07
		M10	350	2	6	0	0.02
		M17.5	200	1	4	1	0.03
		M31	326	0	0	0	0
#3	4 Gy	H1	21	4	0	0	0.19
		M2	503	32	8	1	0.08
		M10	500	5	3	0	0.02
		M17.5	250	0	1	0	0.004
		M31	553	0	3	0	0.005
#4	4 Gy	H1	27	9	0	0	0.33
		M2	199	12	3	0	0.075
		M10	500	15	1	0	0.03
		M17.5	450	1	4	0	0.01
		M31	280	0	0	0	0

D+F: Dicentric + fragment
D-F: Dicentric – fragment
Rc+F: Centric Ring + fragment

Table 2

Radiation dose	Animal	Time after irradiation	Number of cells observed	Ttot			TwT		
				Number of Translocations	Frequency (genome equivalent)	Stability**	Number of Translocations	Frequency (genome equivalent)	Stability**
2 Gy	#1	**H1***	**991**	**80**	**0.26**	**reference**	**46**	**0.15**	**reference**
		M2	1,047	71	0.22	ns	56	0.17	ns
		M10	1,478	69	0.15	P<0.05	49	0.1	ns
		M17.5	1,061	51	0.15	P<0.05	32	0.1	ns
		M31	1,518	58	0.12	P<0.05	50	0.1	ns
	#2	**H1**	**977**	**59**	**0.19**	**reference**	**35**	**0.11**	**reference**
		M2	3,020	185	0.195	ns	110	0.12	ns
		M10	3,196	146	0.14	ns	120	0.12	ns
		M17.5	1,041	36	0.11	p< 0.05	24	0.07	ns
		M31	984	35	0.11	p< 0.05	30	0.1	ns
4 Gy	#3	**M2**	**2,257**	**255**	**0.36**	**reference**	**185**	**0.26**	**reference**
		M10	2,594	213	0.26	p< 0.05	163	0.20	p<0.05
		M17.5	1,820	188	0.33	ns	163	0.285	ns
		M31	1,580	167	0.34	ns	142	0.29	ns
	#4	**M2**	**1,103**	**125**	**0.36**	**reference**	**100**	**0.29**	**reference**
		M10	2,016	148	0.23	p<0.05	112	0.18	p<0.05
		M17.5	667	70	0.33	ns	35	0.17	p<0.05
		M31	1,523	139	0.29	ns	119	0.25	ns

*: Abbreviations used: H1: 1 hour after irradiation. M: months after irradiation
**: Chi-square test as compared with the value H1 or M2.
ns. Not significant