INT. J. RADIAT. BIOL 2002, VOL. 78, NO. 9, 845–855

Taylor & Francis
healthsciences

Life span, cancer and non-cancer diseases in mouse exposed to a continuous very low dose of γ-irradiation.

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(Received 25 January 2002; accepted 18 April 2002)

Abstract.
Purpose: To analyse the life-span and pathologies of mice living under a continuous very low-dose γ-irradiation.
Material and methods: We exposed 300 C57Bl/6J female mice, 3 weeks old, to 10 cGy year^{-1} γ-rays while 300 control mice lived in the same room. Irradiation was delivered continuously by thorium nitrate. We kept all the animals until natural death and performed autopsy.
Results: No difference was observed in life-span (mean life-span ± SE: 805.2 ± 9.62 days for controls and 815 ± 9.57 days for irradiated mice), weight curves or food intake. At autopsy, cancer was present in 40.9% of controls and 37.9% of irradiated mice. They were mainly represented by lymphomas (23.7 and 24.9%) and histiocytic sarcomas (12.6 and 8.7%, respectively, for controls and irradiated mice). Vascular diseases occurred in 24.1% of controls and 23% of irradiated mice. Infections were present at autopsy in 14.1 and 12.3%, respectively, of controls and irradiated animals. No statistical difference was observed at the end of the experiment for cancer or non-cancer diseases between the two groups.
Conclusion: Continuous 10 cGy year^{-1} γ-irradiation had no adverse effect on malignant or non-malignant diseases in this strain of mouse.

1. Introduction

The biological effects of low and very low doses of ionizing radiation are still a matter of debate. The possible risks arising from low-dose and low-dose-rate exposure to ionizing radiation are central to the setting of standards for radiological protection. To date, epidemiology provides the data from people used by those regulating risk assessment with cancer incidence being considered as the main end-point. In the Japanese A-bomb survivors cohort, it is still not clear whether there is an increased risk of cancer and especially leukaemia at very low doses (Pierce *et al.* 1994, Little and Muirhead 1998, 2000). Other studies performed on occupationally or environmentally exposed groups are currently under progress (Cardis *et al.* 2001) and they should be more informative for the direct estimation of the effects of very low doses of radiation. Unfortunately, the inherent limitations of epidemiology for small doses (< 100 mSv) make it extremely difficult to quantify health risks from these exposures.

Animal models represent a useful tool to analyse the biological effects of low-dose exposure since the nature of ionizing radiation, the schedule of administration, the dose and dose-rate can be easily modelled. After the first experiments of Lorenz *et al.* (1955) showing a significant increased life-span of the LFA1 male mouse daily irradiated with 0.11 cGy γ-rays, other animal studies have also reported either an absence of adverse effects or a beneficial (also called hormetic) effect using single or protracted very low doses of radiation. For example, Maisin *et al.* (1996) described a significant increase of life-span in the C57BL/Cnb mouse exposed to a single dose of 50 cGy X-rays. However, no significant modification of life-span was observed on the BC3F1 mouse after single doses of 4–32 cGy X-rays (Covelli *et al.* 1988), on the C57Bl/Cnb mouse with fractionated doses of γ-rays < 1 Gy (Maisin *et al.* 1988) or on mouse exposed to 0.8 cGy day^{-1} γ-rays for 1 year (Bustad *et al.* 1965). Thompson *et al.* (1985) described no detrimental effect on life-span on the B6CF1 mouse exposed to single doses of γ-rays of 22.5 and 45 cGy, while there was a shortening of life with 90 cGy.

The rate of ovarian tumours, mammary and lung adenocarcinomas was not modified in the BALB/c mouse after a single administration of 10 cGy, while it increased significantly after 25 cGy γ-rays (Ullrich 1983). In contrast, the AKR mouse, a strain which develops spontaneous thymic lymphomas, showed a significantly lower incidence of tumours after fractionated γ-irradiation with two or three weekly doses of, respectively, 15 or 5 cGy (Ishii *et al.* 1996).

We have previously shown that the female C57Bl/6 mouse continuously irradiated for its whole life with dose-rates as low as 7 or 14 cGy year^{-1} γ-rays had a significantly higher mean life-span when compared

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with controls living in the same room (549 ± 9 days for controls, 673 ± 13 days for both irradiated groups) (Caratero *et al.* 1998). To understand the mechanisms involved in this biological effect, we conducted a new experiment using the same strain of mouse. They were irradiated with a single intermediate dose-rate of 10 cGy year^{-1} as we did not observe any difference between the two irradiation rates. To date, no experimental data are available on either tumour or non-tumour incidences using such chronic irradiation. Longevity, body weight and pathologies presented at autopsy were recorded.

2. Materials and methods

2.1. *Mouse*

One thousand pathogen-free C57BL/6 female mice, 3–4 weeks old, were purchased from Iffa Credo (l'Arbresle, France). They were separated into two groups (500 mice each): controls and irradiated. In each group, 300 mice were kept for a longevity experiment while 200 were killed at different times for experimental purposes. They were housed in a room lit from 07:00 to 19:00 hours, kept at 19–21°C with a relative humidity of 60% ± 15%. Food and sterile water were provided *ad libitum*. The mice were housed 20 per cage and kept until natural death for the longevity experiment. The cages were placed on three-step racks. In order to place all the mice from a group in the same conditions, rotation of the cages was done weekly. The caloric intake per cage was estimated by weighing the food given and the food remaining in each cage every week. Animal care consisted of weekly cleaning of the cages and bottles and changing sawdust litter, daily checking of the condition of the animals and the use of germ-limited conditions by the keeper (gloves, gown, covered hair). When animals seemed sick or moribund, they were kept in isolation until natural death or recovery. Body weights were recorded monthly. An animal care committee approved the experiment.

2.2. *Irradiation and dosimetry*

Irradiation was given by thorium nitrate. This natural radio-emitter was chosen to allow a comparison with a previous experiment (Caratero *et al.* 1998). ^{232}Th emits γ-rays of mean 60 KeV energy and α-rays of 4 MeV energy. Its half-life of 1.4×10^{10} years ensured a constant rate of irradiation during whole experiment. Thorium nitrate powder was housed in airtight plastic bags, which were covered by 25-mm thick chipboard to protect the bags. It also attenuated the level of irradiation to keep it to the required dose of 10 cGy year^{-1} γ-rays while stopping α-rays. The cages were placed on the chipboard. Control mice were housed in the same room, 3 m away from the irradiated mice, and isolated by a wooden screen covered by a 1.5-mm-thick sheet of lead.

2.3. *Spectrometry*

The energy spectrum of the radioactive source used for the experiment was measured by using an NaI crystal scintillator 15 cm in diameter and 10 cm in thickness and a 1024-channel analyser. The sample was placed 14 cm from the detector. The spectrum was integrated for 1 h. These measurements were performed at the CESR (Centre d'Etudes Spatiales des Rayonnements, Toulouse, France).

2.4. *Dosimetry*

Thermoluminescent detectors were placed at three different levels inside a polycarbonate cage, especially dedicated to the dosimetry measurements. This cage was placed with the experimental mouse cages. We used $CaSO_4$ powder, housed in a cylindrical plastic container. The exposure duration was for 1 month, and the values obtained were extrapolated to 1 year. Philitech Co. (Jouy en Josas, France) processed the dosimeters.

2.5. *Histopathology*

As we chose to wait for the natural death of the mice, some of the animals were excluded from the autopsy study when post mortem decomposition was too far advanced. Thus, in order to increase the number of animals in the autopsy group, other mice living in the same conditions, but which were attributed to experimental design, were included when natural death occurred.

Macroscopic examination was performed. The following organs were systematically removed when present (cannibalism was frequent by the other mice in the same cage): lungs, heart (as a whole), salivary glands, liver, ovaries, kidneys, spleen, adrenal glands, thymus, lymph nodes, mammary glands and brain. They were fixed in Bouin's solution, except the brain, which was fixed in 10% formalin for 1 week. After paraffin embedding, 5-μm sections were prepared and the slides stained with both haematoxylin and eosin (H&E) and Masson's trichrome (which stains collagen fibres blue). Brains were only analysed when death was recent because of the rapid destruction of its microscopic structure. Three sections including

frontal cortex, parietal cortex and cerebellum were systematically prepared.

The slides were examined by two pathologists blind to the exposure status. As it is sometimes difficult to prove that a specific lesion is the sole cause of the death, we recorded the diseases present at autopsy. Thus, in some mice, when two pathologies were present at autopsy, they were both taken into account. When the cause of death was uncertain, no diagnosis was made.

2.6. *Immunohistochemistry of lymphomas*

To classify the different subtypes of lymphoid tumours by light microscopy and to establish the differential diagnosis with reactive lymph nodes, we applied immunohistochemistry on deparaffinized sections. The polyclonal anti-CD3 antibody (A0452, Dako, Golstrup, Denmark) and the monoclonal antibody B220 (anti-CD45R clone RA3-6B2, Pharmingen, BD, Le Pont de Claix, France) were used for the detection of T- and B-lymphocytes respectively, and combined with biotinylated goat anti-rabbit IgG or biotinylated rabbit anti-rat IgG, respectively, (Dako). Avidin–biotin peroxidase complex was thus applied followed by visualization with 3,3′-diaminobenzidine (all Dako). Haematoxylin was used as a counterstain. The criteria for the histological phenotypes of lymphoid tumours were: T-cell lymphomas when the malignant lymphocytes were CD3+ cells, B-cell lymphomas when cells were B220+. Not infrequently, B-cell lymphomas were mixed with reactive T-cells (identified by a CD3+ staining of small lymphocytes). B-cell lymphomas were separated into lymphoblastic–lymphocytic, follicular centre cell or plasma cell lymphomas. Reactive lymph nodes were diagnosed when B- and T-lymphocytes where present (even if hyperplastic) in their usual place in the lymph nodes and/or in the spleen.

2.7. *Statistics*

Differences in the life-span between groups were estimated at the end of the experiment by comparison of average life-spans. Comparison of the total number of mice bearing specific pathologies at the end of the experiment was made using Fisher's exact test (two-sided). Kapplan–Meier survivor analysis was used to estimate survival of mice from both groups with specific pathologies and the survival probability is represented as a function of time. The logrank (Mantel–Cox) test was used to test for significant differences in these survival curves and p's are given. Statistical analysis was performed using DM90 Software.

3. Results

3.1. *Spectrometry*

Calibration of the detector led to the transfer function given by:

$$E\,(\mathrm{KeV}) = (3.288 \times \mathrm{channel}) - 65.503,$$

$$\text{with } R^2 = 0.999.$$

The background contribution has been removed from the measurement spectrum. The spectrum plotted in figure 1 was thus entirely due to thorium nitrate. For easier reading, counts were multiplied by 10 from channels 400–1024. Measurements performed on the sawdust used for the mouse litter and on the chipboard failed to detect any radioactivity contribution.

3.2. *Dosimetry*

The results showed a dose gradient inside the cage:

- 11.28 cGy year^{-1} at the bottom of the cage (floor).
- 8 cGy year^{-1} at the food support level.
- 6 cGy year^{-1} at the top of the cage (ceiling).
- 0.35 cGy year^{-1} for the control set without radioactive source.

As the mice mainly move around the floor, the sawdust- and the food-support level in a cage, we estimated the average dose-rate to be 10 cGy year^{-1}.

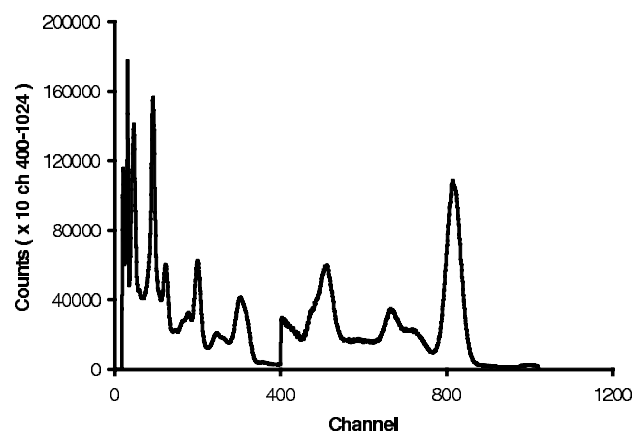


Figure 1. Energy spectrum of the source. The detector was placed 14 cm from the source and the spectrum was integrated for 1 h. Background has been subtracted. For channels 400–1024, counts have been multiplied by 10 for easier reading.

3.3. *Life-span*

Survival curves are shown in figure 2. Curves of controls and irradiated groups remained close throughout the experiment. The mean life-span was 805.2 ± 9.62 and 815 ± 9.57 days, respectively, for control and irradiated mice. The maximum life-span was the same in the two groups, 1048 days. Even though the survival curve of irradiated mice was above the control curve, the difference was not statistically significant ($p = 0.54$).

Body weight curves are shown in figure 3. As expected, the mean body weights increased until 20 months of age, and then decreased. No difference was observed between the two groups of mice. There was also no difference between groups in calorie intake (data not shown).

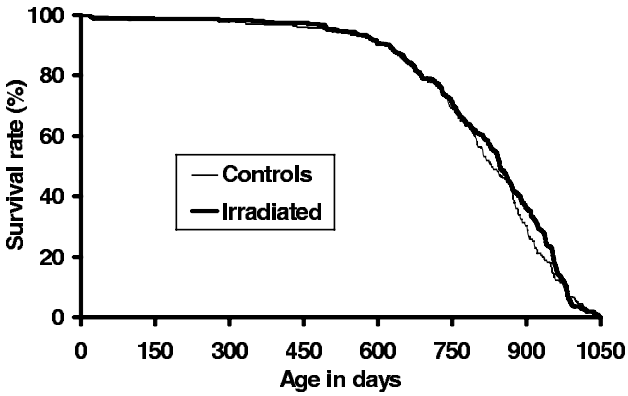


Figure 2. Survival curves. Each group contained 300 mice at the start of the experiment.

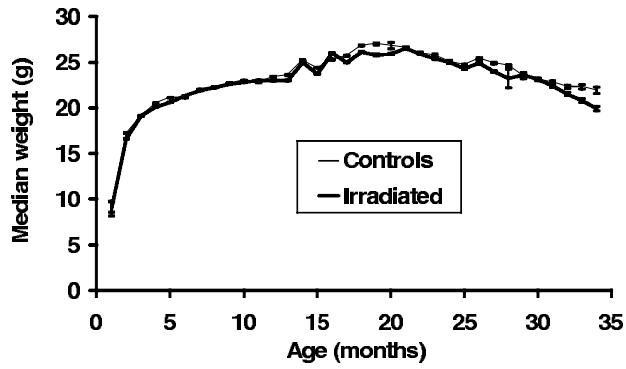


Figure 3. Body weight data. Each point is the mean weight ± SE of surviving animals.

3.4. *Histopathology*

3.4.1. *Histopathologic criteria.* Pathologies were separated into tumour and non-tumour categories. Tumours were classified as benign or malignant. The main pathologies encountered are shown in figure 4.

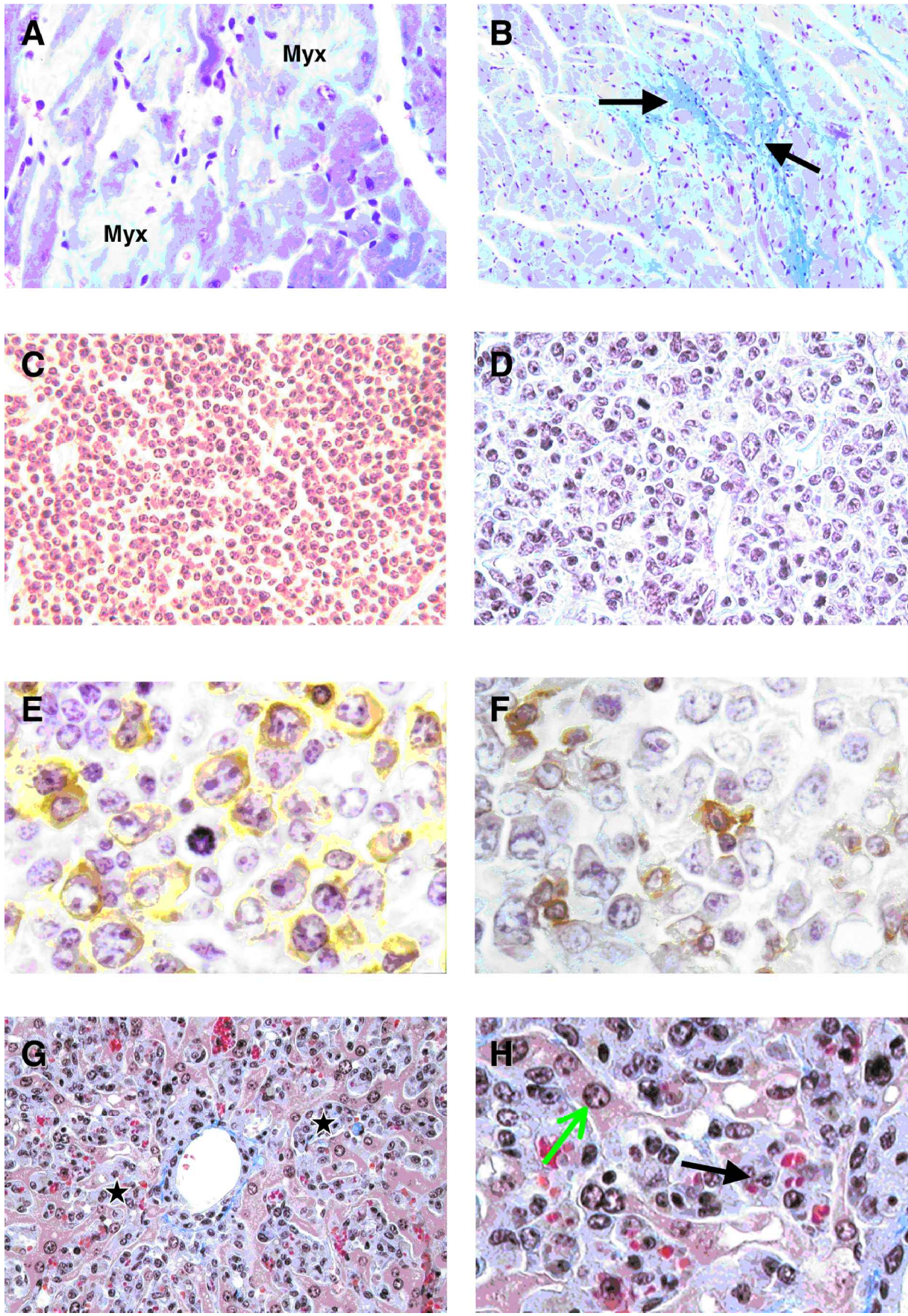
3.4.1.1. *Malignant tumours*

3.4.1.1.1. *Haematologic tumours.* Lymphomas correspond to tumours developed from lymphocytes. They usually appear in lymph nodes or thymus and then may disseminate to other organs. They were diagnosed when there was an enlargement of lymph nodes (whatever their location) and/or thymus, related microscopically to a proliferation of small, normal (figure 4C) or atypical lymphoid cells (figure 4D) with destruction of the normal architecture. Generally, at autopsy the lymph nodes and/or the thymus were enlarged, as sometimes was the spleen. Microscopically, on occasions other organs were involved such as the liver, kidneys or lungs. They were separated into B (figure 4E, F) and T-cell lymphomas and into lymphocytic–lymphoblastic (proliferation of uniform small lymphocytes) or pleomorphic (proliferation of follicular centre cells or plasma cells) lymphomas according to Jones *et al.* (1990).

Histiocytic sarcomas (formerly called reticulum cell lymphoma, Dunn type-A) represent a tumour derived from histiocytic cells, and probably from the Kupffer's cells in the liver (Turusov 1994). This neoplasm was suspected at autopsy when there was an ascites-associated liver enlargement. The uterus was very seldom involved. It was confirmed microscopically by features of proliferation of histiocytic cells with nuclear atypia. These neoplastic cells appeared sometimes focally in the liver but frequently there was a diffuse enlargement with many tumour cells in the liver sinusoids (figure 4G, F). The same neoplastic cells were also observed in other organs (mainly lungs or the spleen) and considered as metastases.

Leukaemias were not observed.

Figure 4. Morphological features of the main pathologies observed at autopsy. (A) Myxoid degeneration of the heart appears as pale blue areas (Myx) replacing myocardic fibres (Masson ×1000). (B) Myocardial infarction appears as a fibrous scar (arrows) in the place of myocardial fibres (Masson ×400). (C) Lymphocytic lymphoma cells are small and have a monomorphic appearance (haemalum & eosin ×400). (D) Follicular centre cell lymphoma cells are of medium size and display nuclear irregularities (Masson ×400). (E, F) Follicular centre cell lymphoma, immunohistochemistry; lymphoma cells are stained with B220 antibody (E) and reactive T-cells (CD3+) are also present (F). (G) Histiocytic sarcoma (Masson ×400); tumour cells invading liver sinusoids appear in blue (stars) and are close to the centolobular vein in the centre of the photograph. (H) Histiocytic sarcoma; same case as in (G) with a higher magnification (Masson ×1000); tumour cells (black arrow) display nuclear irregularities and are smaller than hepatocytes (green arrow).



3.4.1.1.2. *Non-haematologic tumours*. Sarcomas correspond to tumours derived from cells of mesenchymal origin. They were diagnosed as proliferations of spindle-shaped cells with nuclear atypias associating either necrosis or mitotic figures. They included tumours developed from the subcutaneous tissue (fibrosarcomas), the bone (osteosarcomas) or the digestive tract (leiomyosarcomas).

Other observed malignant tumours observed were adenocarcinomas of the lungs and hepatocarcinomas.

Only a few tumours remained of unknown origin.

3.4.1.2. *Benign tumours*. Fibromas were diagnosed when there was a proliferation of spindle-shaped cells without nuclear atypias, necrosis or mitotic figures.

Pituitary adenomas appeared after the 30th month. They appeared as an enlargement of the pituitary gland microscopically related to proliferation of cubical cells lined by blood vessels. As the brain was kept only when death was recent, we have probably underestimated the frequency of pituitary adenomas.

Other benign tumours included adenomas or focal nodular hyperplasia (also called hepatomas) of the liver.

3.4.1.3. *Non-tumour diseases*

3.4.1.3.1. *Vascular diseases*. Myocardial infarction was evoked on the Masson's stain of the hearts in the presence of fibrous scars in place of the myocardial fibres (figure 4B), with or without infiltration of polymorphonuclears or coronary obstruction. Myocardial myxoid degeneration was also diagnosed on Masson's stains of the hearts as blue myxoid areas instead of myocardial fibres (figure 4A). Mesenteric infarction was diagnosed macroscopically and confirmed microscopically by the necrosis of the total lining of the intestine. Venous thrombosis or acute pulmonary oedema were also observed.

3.4.1.3.2. *Infections*. Acute infections were noted microscopically when we observed collected infiltrates of polymorphonuclear cells (abscess) whatever the organ involved (kidney, salivary glands, lung, cardiac valve, etc.). Chronic infections were diagnosed when there was an association of a chronic cutaneous wound, spleen enlargement related to sclerosis and liver haematopoiesis.

Congenital malformations of vascular origin (haemangiomas) were diagnosed in some mice in various organs but mainly in the liver or the spleen.

3.4.2. *Results of the autopsy study*. The results of the autopsy study are summarized in table 1. It was performed on 325 controls and 309 irradiated mice.

Table 1. Results of the autopsy study.

	Controls	Irradiated
Number of animal autopsied	325	309
All malignancies	*133 (40.9%)*	*117 (37.9%)*
Haematopoietic malignancies	*118 (36.3%)*	*104 (33.7%)*
Histiocytic sarcomas	41 (12.6%)	27 (8.7%)
Lymphomas	77 (23.7%)	77 (24.9%)
Malignant tumours (non-haematopoietic)	*15 (4.6%)*	*13 (4.2%)*
Sarcomas	10 (3.1%)	11 (3.6%)
'Others	5 (1.5%)	2 (0.6%)
Benign tumours	*10 (3.1%)*	*9 (2.9%)*
Pituitary adenomas	6 (1.8%)	6 (1.9%)
Others	4 (1.2%)	3 (0.1%)
Vascular pathologies	*79 (24.3%)*	*71 (22.3%)*
Myocardial infarction	40 (12.3%)	34 (11%)
Heart myxoid degeneration	30 (9.2%)	24 (7.8%)
Mesenteric infarction	5 (1.5%)	7 (2.3%)
Others	4 (1.2%)	6 (1.9%)
Infections	*46 (14.1%)*	*38 (12.3%)*
Acute	14 (4.3%)	14 (4.5%)
Chronic	32 (9.8%)	24 (7.8%)
Vascular malformations	*9 (2.8%)*	*8 (2.6%)*

The main diseases observed at autopsy were haematopoietic malignancies, infections (acute and chronic) and vascular diseases. Malignant diseases were present in 40.9% of controls and in 37.9% of irradiated mice (Fisher's exact test, two-sided, with $p = 0.33$). The other pathologies had a lower prevalence. Even though the rate of benign and malignant diseases was always lower in the irradiated group, there was no statistical difference in any pathology rate at the end of the experiment. More interestingly, we observed that lymphomas and histiocytic sarcomas seemed to occur later in irradiated mice than in controls. We have, therefore, represented in figures 5–7 Kapplan–Meier estimates of survey with the main diseases present at autopsy as a function of time.

The total number of haematopoietic malignancies, lymphomas or histiocytic sarcomas was not statistically different between groups at the end of the experiment (Fisher's exact test, two-sided, with $p = 0.45$, 0.71 and 0.09, respectively). There is a suggestion of a delay in appearance of these pathologies in irradiated mice when compared with controls (figure 5) but the difference was not significant (log-rank test with $p = 0.30$, 0.47 and 0.40, respectively). It was interesting to observe that the delay in appearance of lymphomas in irradiated mice disappeared at the end of their life (figure 5B). The percentage of T- and B-cell lymphomas and their subtypes are indicated in table 2. B-cell lymphomas accounted for, respectively, 90 and 84% in controls and irradiated

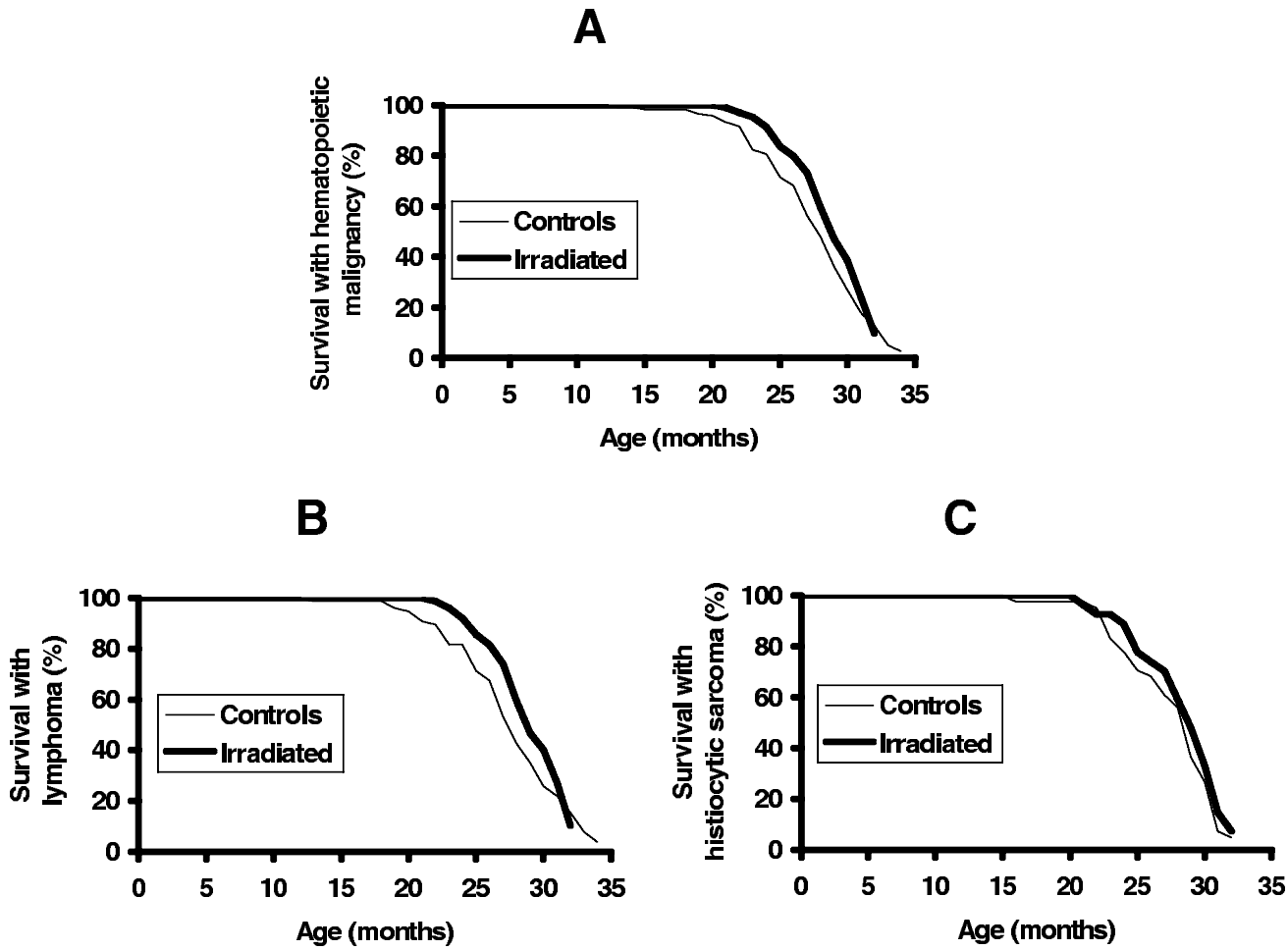


Figure 5. Kapplan–Meier estimates for survival of mice with evidence of haematopoietic malignancy (A), or with either lymphoma (B) or histiocytic sarcoma (C), comparing the control and irradiated groups. No significant differences were observed between groups, logrank (Mantel–Cox) test with, respectively, $p = 0.31$ (A), 0.47 (B) and 0.40 (C).

Table 2. Immunohistochemical classification of the lymphomas.

		B-cell lymphomas		
	T-cell lymphomas	Lymphoblastic lymphocytic	Follicular centre cell	Plasma cell
Controls	9.6	23.1	65.4	1.9
Irradiated	16	18	62	4

Data are percentages.

mice. There was no statistical difference between groups for these subtypes.

Infection rates were not statistically different between groups (Fisher's exact test, two-sided with $p = 0.55$), even if the total rate of chronic infections was lower in the irradiated group (table 1). Survival curves of animals with infection (acute or chronic) (figure 6A) or chronic infection alone (figure 6B) were closely the same in both groups (logrank test with $p = 0.84$ and 0.95, respectively).

The total number of vascular diseases was slightly lower in irradiated mice (22.9 versus 24.3% in controls) but without statistical difference (Fisher's exact test, two-sided with $p = 0.57$). Survival curves of animals with vascular disease (figure 7A) or myocardial infarction alone (figure 7B) were nearly the same, without statistical difference (logrank test with $p = 0.82$ and 0.61, respectively).

The incidence of the other types of tumours was very low and no differences were observed between

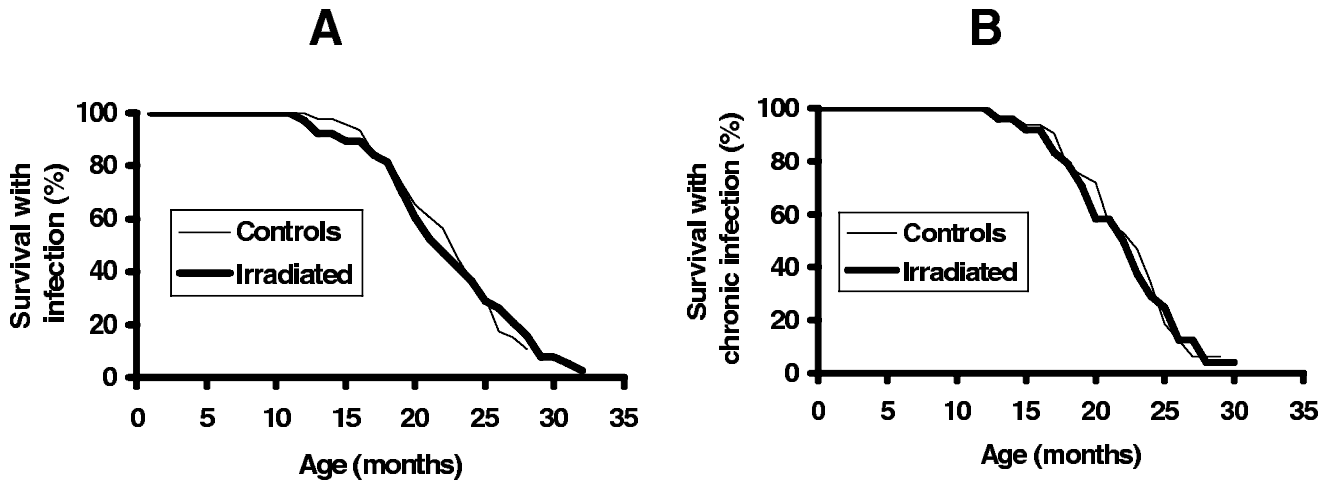


Figure 6. Kapplan–Meier estimates for survival of mice with infection (acute or chronic; A) or chronic infection alone (B) comparing the control and irradiated groups. No significant differences were observed between groups, logrank (Mantel–Cox) test with $p = 0.84$ and 0.96, respectively.

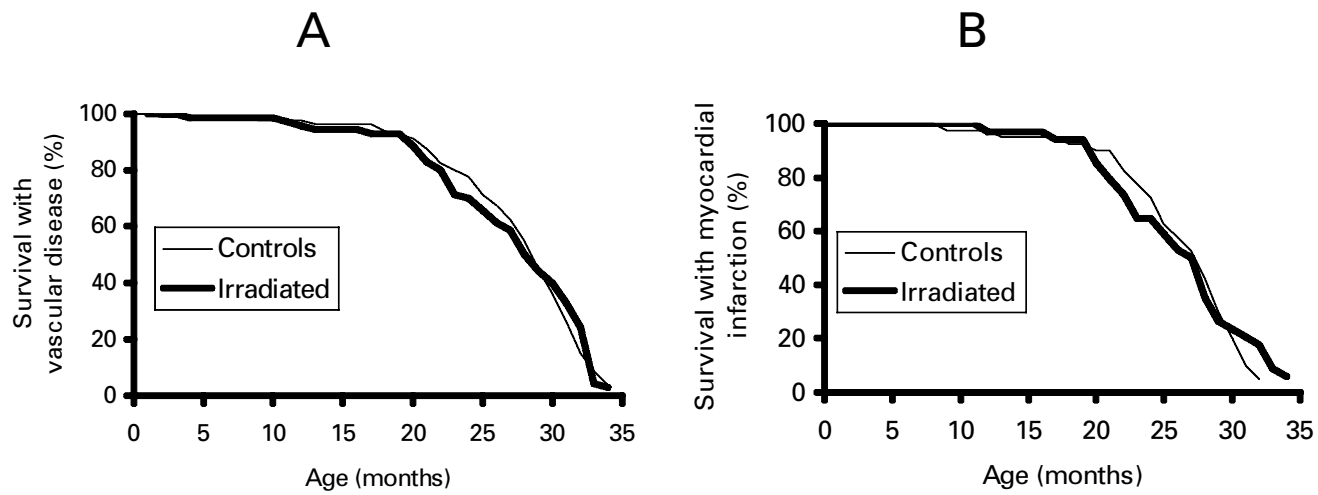


Figure 7. Kapplan–Meier estimates for survival of mice with evidence of vascular disease (A) or myocardial infarction alone (B) comparing the control and irradiated groups. No significant differences were observed between groups, logrank (Mantel–Cox) test with, respectively, $p = 0.82$ (A) and 0.61 (B).

groups for sarcoma, tumours of undetermined origin or benign tumours.

4. Discussion

We report here for the first time the rate of pathologies present at autopsy in mouse exposed to a chronic very low-dose γ-irradiation. We conclude that 10 cGy year^{-1} γ-irradiation has no harmful effect on C57Bl/6J mice.

We did not observe an increase in the mean life-span of irradiated mice when compared with controls as described in Caratero *et al.* (1998). Moreover, in this experiment, the total life-span was increased in both groups in comparison with our previous work (805 versus 549 days for controls, 815 versus 673 days for irradiated mice). This is probably due to a modification of the experimental housing of the mice. In this experiment, they were kept 20 per cage instead of 30 previously and we applied germ-limited conditions to avoid infections. It has been shown that housing conditions can influence life-span. For example, rats housed individually lived longer than rats housed four per cage (Skalicky *et al.* 2001). The mean life-span of the mice in this experiment is similar to that observed by other authors (798–820 days) (Kunstyr and Leuenberger 1975, Blackwell *et al.* 1995, Babitt *et al.* 2000).

In our previous experiment, the control mice began to die after 8 months of life. As we observed in this study that lymphomas began to appear after 20 months of life (figure 5B), it is unlikely that the

controls from the first experiment died of lymphomas. Moreover, in the previous experiment (unpublished data) we observed that control mice developed more skin infections than did the irradiated animals. In this latter experiment, these infections were not observed mostly due to the particular care taken to avoid them. Thus, the early mortality described in the controls from the first experiment must have been due to skin infectious diseases which were favoured by the great number of animals in the same cage, the irradiated mice being 'protected'. In this experiment, we noted a lower number of infectious diseases (table 1) in the irradiated group at the end of the experiment, even if the difference was not significant. This result is in good accordance with decreased levels of some immunoglobulins observed in the irradiated mice (Courtade *et al.* 2001), which can be attributed to a lower antigenic stimulation. Indeed, Lorenz *et al.* (1955), who were the first to describe an increase of the mean life-span of rodents under chronic irradiation (0.11 cGy day^{-1} during their whole life), considered this effect in the light of a decrease of the rate of infectious diseases. It has been suggested that they would not have observed this result if they had used pathogen-free animals (Congdon 1987). This would mean that the hormetic effect is greater observed under stressing conditions.

The absence of modification of the weight curves and of the food intake attests that this chronic low-dose does not act through a reduction of calorie intake. This is noteworthy as, to date, calorie restriction is the only known way to increase the total life-span (Blackwell *et al.* 1995) through retarding ageing pathologies (Masoro 2000).

Irradiation used here had no adverse effect on the rate of vascular pathologies and especially on heart infarction (figure 7). An accelerated occurrence of vascular pathologies has been reported in Chernobyl liquidators (Polyukhov *et al.* 2000), and there was a significant increase of heart diseases mortality among Japanese A-bomb survivors (Shimizu *et al.* 1999).

The rate of non-haematologic tumours was very low in this strain of mouse. In contrast, haematologic tumours represented the main cause of death. Their frequency is in good accordance with those described by Babitt *et al.* (2000), Boorman *et al.* (2000) and Turusov (1994). The total rate of haematologic tumours and especially of histiocytic sarcomas was lower in the irradiated group (table 1), but the difference was not significant ($p = 0.45$ and 0.09, respectively). There was also a tendency for the delayed appearance of both lymphomas and histiocytic sarcomas in irradiated mice (figure 5B, C), but without statistical difference. It was interesting to observe that this tendency disappeared for the lymphomas at the end of the life as the rate reached the control levels about the 31st month of life.

An absence of modification of tumour rate after irradiation has already been reported in different strains of mice. For example, Maisin *et al.* (1988) described an significant increased rate of tumours in C57Bl/Cnb mice with doses of γ-rays > 2 Gy, while no difference was noticed with doses of 25 or 50 cGy. Covelli *et al.* (1988) reported a lower but not significant rate of lymphomas with a 4 cGy X-irradiation, while no difference was observed with 8, 16, 32 or 64 cGy in $BC3F_1$ mice. Histiocytic sarcomas were significantly less frequent in the F1 progeny of C57Bl/6 male mice irradiated with a dose of 3 Gy 15 days before mating with unirradiated females (Iwasaki *et al.* 1996). Babitt *et al.* (2000) also reported a lower rate of histiocytic sarcomas in mouse irradiated with 3, 4 or 5 Gy γ-rays.

Interestingly, the latent period but not the frequency of radiation-induced leukaemia was significantly increased in CBA/H mice given a pretreatment with an adaptive dose of 10 cGy γ-rays 24 h before a 1 Gy dose that induced acute myeloid leukaemia (Mitchel *et al.* 1999), as found for lymphomas in our experiment (figure 5B). According to these authors, the adaptive response indicates that earlier exposure to a small dose of radiation can influence secondary steps in radiation-induced carcinogenesis. Other reports indicate that low-dose irradiation depletes carcinogenic potential in mouse (reviewed by Bhattacharjee and Ito 2001) and doses as low as 1 cGy 24 h before inoculation of lymphoma cells in mouse are sufficient to delay and reduce tumour size. We can hypothesize that our 10 cGy $year^{-1}$ irradiation initiates an adaptive response that modifies the steps of carcinogenesis of naturally occurring lymphomas in C57Bl/6J mice, this beneficial effect disappearing at the end of life. Indeed, the adaptive response was abolished in lymphocytes from elderly donors, suggesting that ageing could be a factor that abolishes the adaptive response (Gadhia 1998). The steps of development of lymphomas and histiocytic sarcomas are still unclear and involve various genes (Okumoto *et al.* 1990).

Concerning the lymphomas, their features are close to those observed in humans and their pathogeny has been quite extensively studied, but it is still unclear. An increased rate of mutations of the *Kras* gene has been described after a 2-Gy γ-irradiation in T-cell lymphomas of Scid mice but the rate of mutation remained relatively low (Nishimura *et al.* 1999). Recently, the analysis of the development of spontaneous germinal centre derived B-cell lymphomas in C57L/J mice suggest that it is similar, if not identical, to those of SJL mice. In this latter

strain of mice the mouse endogenous mammary tumour virus superantigen (Mtv-vSAgs) and Vβ16 T-cell stimulation play an important role in their development (Sen *et al.* 2001). Moreover, the final outcome of the spontaneous neoplastic process appears strongly influenced by endogenous natural killer activity in ageing mice (Erianne *et al.* 2000). Unfortunately, no data are available on the factors influencing the appearance of B-cell lymphomas in C57Bl/6J mice, but they lack Mtv-7 superantigen (Desaymard *et al.* 1993) suggesting a different way of lymphomagenesis. Only 65% of lymphomas observed at autopsy (table 2) in our study were germinal centre-derived B-cell lymphomas like those described in SJL mice.

Histiocytic sarcomas are developed from cells of the phagocyte system. Such tumours are rather specific to rodents and they are very rare in humans. An increased rate of this tumour has been described in CBA mice after treatment with diethystilbestrol or in mice submitted to repeated antigen stimulation (Turusov 1994). Our observation of fewer chronic infections in irradiated mice is perhaps an explanation of its lower rate of occurrence in this group. We also noticed the frequent association between histiocytic sarcoma and liver haematopoiesis: 80% of the cases were associated with liver haematopoiesis compared with 40% in non-tumour bearing mice (data not shown). Histiocytic sarcomas should preferentially develop in the liver with haematopoietic activity, but can such haematopoiesis be considered physiological or does it reflect a dysfunction of the bone marrow? Studies are in progress to clarify this point.

In conclusion, we have shown that 10 cGy year^{-1} γ-irradiation had no adverse effect on life-span, cancer or non-cancer rate on C57Bl/6J mice. Complementary *in vitro* studies are in progress to test whether such a continuous and very low-dose irradiation can really initiate an adaptive response.

Acknowledgements

The authors thank D. Cerezo, C. Reyes, V. Blanco and R. Destrade for technical assistance. This study was supported by research grants from Electricité de France and Conseil Régional Midi-Pyrénées.

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