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Activation of immunological network by chronic low-dose-rate irradiation in wild-type mouse strains: Analysis of immune cell populations and surface molecules

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Abstract

Purpose: To analyse the effects of chronic whole body low-dose-rate irradiation on the immune system in various wild-type mouse strains in comparison with the effects from acute high-dose-rate irradiation.

Materials and methods: Wild-type mouse strains (C57BL/6, BALB/c, C3H/He, DBA/1, DBA/2 and CBA) were observed after chronic low-dose-rate γ irradiation at 1.2 mGy hour^{-1} by intensive analysis of immune cell populations and their various surface molecules, together with antibody-producing activity both with and without immunization by sheep red blood cells (SRBC). The cell surface functional molecules [cluster of differentiation (CD) 3, CD4, CD8, CD19, CD45R/B220, intercellular adhesion molecule (ICAM)-1, Fas, natural killer (NK)-1.1, chemokine {C-X-C motif} receptor 4 (CXCR4) and chemokine {C-C motif} receptor 5 (CCR5)] and activation molecules [thymocyte-activating molecule (THAM), CD28, CD40, CD44H, CD70, B7-1, B7-2, OX-40 antigen, cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), CD30 ligand and CD40 ligand] were studied in the bone marrow, thymus, spleen, lymph nodes and peripheral blood by flow cytometry.

Results: By chronic low-dose-rate irradiation alone, CD4+ T cells and CD8 molecule expression increased significantly by a maximum of 30%, while CD40+ B cells decreased significantly. Increases of CD4+ T cells, CD40+ B cells and anti-SRBC antibody-producing cells by immunization were significantly enhanced by continuous low-dose-rate irradiation at 1.2 mGy hour^{-1}. CD3− CD4+ T cells, representative of abnormal immune cells, were absent in the chronically low-dose-rate-irradiated mice, while a dose-dependent increase of these cells was found in acutely high-dose-rate-irradiated mice given the same total doses.

Conclusion: Chronic low-dose-rate radiation activated the immune system of the whole body.

Keywords: *Chronic low-dose-rate irradiation, immunological network activation, immune cell populations, cell surface molecules, abnormal immune cells, wild-type mouse strains*

Introduction

A high dose of ionizing radiation, given acutely at high dose-rate, is generally known to be harmful to living organisms. However, high-dose-rate, low-dose radiation can induce various biological activities in living organisms, including activation of immune functions (Anderson & Lefkovits 1979, Liu et al. 1987, James & Makinodan 1988, James et al. 1990, Matsubara et al. 2000, Kojima et al. 2000, 2002, Cheda et al. 2004), prevention and cure of disease (Dobbs et al. 1981, Jacobs & King 1987, Shen et al. 1997, Hashimoto et al. 1999, Takahashi et al. 2000, Ishii et al. 1996, Mitchel et al. 1999, 2003, 2004), augmentation of growth rate (Luckey 1991), increase of resistance to oxygen toxicity (Lee & Ducoff 1989), enhancement of survival after lethal high-dose-rate irradiation given at high dose (Yonezawa et al. 1990, 1996) and prolongation of life span (Lorenz et al. 1955, Ducoff 1975, Mine et al. 1990). Low-dose radiation activates proliferative responses of spleen cells to phytohaemaglutinin (PHA) (James & Makinodan 1988, James et al. 1990), concanavalin A (ConA)-induced proliferation of spleen cells, and activity of NK cells both accompanied with elevation of glutathione (Kojima et al. 2000, 2002) and activity of NK cells with suppression of tumour metastases (Cheda et al. 2004).

Recently, we reported an immune activation by chronic low-dose-rate γ irradiation at 1.2 mGy

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hour^{-1} for 3 weeks in C57BL/6 mice (Ina & Sakai 2003). In addition we observed a marked prolongation of the life span of MRL-*lpr/lpr* mice with multiple severe diseases by 5-weeks γ irradiation at a low dose-rate of 0.35 mGy hour^{-1}, more effectively at 1.2 mGy hour^{-1} (Ina & Sakai 2004), and even further prolongation by life-long irradiation at 1.2 mGy hour^{-1} (Ina & Sakai 2005). These life-span prolongations were associated with immunological activations and ameliorations of the severe diseases in the whole body (Ina & Sakai 2004, 2005). Furthermore, radiation-induction of thymic lymphomas by acute high dose radiation given at high-dose-rate was suppressed by pretreatment with low-dose radiation of 75 mGy (adaptive response in tumour induction), and further repressed by life-long irradiation at a low dose-rate of 1.2 mGy hour^{-1}, where this continuous irradiation alone did not produce thymic lymphomas or other tumours (Ina et al. 2005).

In this study we examined the effects of chronic low-dose-rate irradiation on the immune system in various wild-type mouse strains in comparison with the effects from acute high-dose-rate irradiation.

Materials and methods

Animals

Female C57BL/6N Crj, BALB/cAnN Crj, C3H/HeN Crj, DBA/1JN Crj, DBA/2N Crj and CBA/JN Crj mice (5 weeks old) were purchased from Charles River Japan (Yokohama, Japan), and were kept under specific-pathogen-free (SPF) conditions. All the animals were maintained on a light schedule from 7:00 to 19:00 (h) and were fed a standard mouse diet CE-2 (Clea Japan, Tokyo, Japan) with water allowed *ad libitum*. The study was reviewed by the Institutional Animal Care and Use Committee, and the mice were treated in accordance with the governmental guidelines and the guidelines of the Central Research Institute of Electric Power Industry (CRIEPI). The mice in all groups of each strain used had nearly equal body weights which were within 1 standard error at the start of the experiments. All mice of each strain used were taken into the laboratory and put in cages, five to six mice per cage at the age of 5 weeks (day 0).

Irradiation

For acute total-body irradiations at a high-dose-rate, X rays were generated by a 300 kV generator (Model MBR-320R, Hitachi Medical, Tokyo, Japan) at 10 mA with filters of 1.0 mm aluminum (Al) and 0.5 mm copper (Cu) at a dose-rate of 1.6 Gy min^{-1} at the mouse holder position as measured by a built-in ionization chamber. The mice were irradiated at doses of 0 Gy, 0.05 Gy, 0.10 Gy, 0.25 Gy, 0.50 Gy, 0.75 Gy and 1.0 Gy for analysis of immune activity, and were irradiated at doses of 0 Gy, 1.0 Gy, 3.0 Gy and 5.0 Gy for detection of cluster of differentiation (CD) 3− CD4+ T cells (Janeway Jr et al. 2001) at the same high dose-rate at 5 weeks of age. The control mice for these acute irradiations were sham-irradiated at the same age in the irradiation chamber.

Continuous total-body irradiations with low-dose-rate γ rays were carried out in a clean irradiation room equipped with a 370 GBq ^{137}Cs γ-ray source (Chiyoda Technol, Tokyo, Japan) at the CRIEPI long-term low-dose-rate irradiation facility. The design of the irradiation facility and the details of dosimetry with an ionization chamber and a glass dosimeter have been described elsewhere (Hoshi et al. 2000). Mouse cages were placed on shelves located 5 m from the source. The mice were irradiated continuously, except for 1 h in the morning, on weekdays from 5 weeks of age, starting on day 0. The dose-rate was 1.2 mGy hour^{-1} as determined from dosimetry with an ionization chamber and a glass dosimeter in 2000. The tissue dose-rate measured with a glass dosimeter that was embedded in a mouse abdomen under the same irradiation conditions was 0.95 mGy hour^{-1} as measured in a separate experiment. For analysis of immune activity, the mice were continuously irradiated for 0, 1, 3, 5, 7, 9, 13 or 17 weeks at the same low dose-rate. The total doses of each irradiation were 0 Gy, 0.2 Gy, 0.6 Gy, 1.0 Gy, 1.4 Gy, 1.8 Gy, 2.6 Gy and 3.4 Gy. Furthermore, for detection of CD3− CD4+ T cells, the mice were also continuously irradiated at the same low dose-rate until the total doses reached to 0 Gy (0 week), 1.0 Gy (5 weeks), 3.0 Gy (15 weeks) and 5.0 Gy (25 weeks). Shelves holding the cages containing the control mice were placed in the same room behind a wall that shielded them from the radiation.

Analysis of immune status

Immunological analysis followed the methods described previously (Ina & Sakai 2003, 2004, 2005, Ina et al. 2005). Immunological changes were examined in cell populations that expressed various immune-specific surface molecules and also by analysis of anti-sheep red blood cell (SRBC) antibody-producing cell populations. CD3− CD4+ lymphocytes, i.e., CD3− CD4+ T cells were counted as a measure of formation of abnormal immune cells (Janeway Jr et al. 2001, Umeki et al. 1997).

Single cell suspensions were prepared from the bone marrow, thymus, spleen, lymph nodes and peripheral blood in Roswell Park Memorial Institute

(RPMI) 1640 medium supplemented with 10% fetal calf serum (FCS). The cell surface functional molecules [CD3, CD4, CD8, CD19, CD45R/B220 (B220), intercellular adhesion molecule (ICAM)-1 (CD54), Fas (CD95), natural killer (NK)-1.1 (NKR-P1, Ly-55), CXCR4 (CD184) and CCR5] and activation molecules [thymocyte-activating molecule (THAM) (CD26, dipeptidyl peptidase (DPP) IV), CD28, CD40, CD44H, CD70, B7-1 (CD80), B7-2 (CD86), OX-40 antigen (CD134), CTLA-4 (CD152), CD30 ligand (CD153) and CD40 ligand (gp39, CD154)] were analysed by flow cytometry.

The acutely high-dose-rate-irradiated mice used for the analysis of immune cell activity were sacrificed or immunized with SRBC after 0 day, 2 days, 1 week, 2 weeks, 3 weeks, 5 weeks and 8 weeks after irradiation; the chronically low-dose-rate-irradiated mice were sacrificed or immunized 0, 1, 3, 5, 7, 9, 13 and 17 weeks after the start of continuous low-dose-rate irradiation. The mice were immunized by intraperitoneal injection of 15% (v/v) SRBC in saline at a dose of 0.02 ml g^{-1} body weight. Saline alone was injected into the control mice. For an analysis of immune activity under a specific antigen condition, the mice were sacrificed 4 days after the SRBC-immunization when the titer of anti-SRBC antibody was at its peak. A single cell suspension was prepared from the spleen in RPMI 1640 medium supplemented with 10% FCS. The numbers of anti-SRBC antibody-producing cells were obtained by counting the plaque-forming cells (PFC).

For an analysis of CD3− CD4+ T cells, acutely, high-dose-rate-irradiated mice and chronically, low-dose-rate-irradiated mice were both sacrificed 10 days after each irradiation. This timing was determined by preliminary experiments to confirm when the cell numbers were at their peak.

Flow cytometry

For immunofluorescence studies, monoclonal antibodies (mAb) from BD PharMingen (San Diego, CA, USA) were used. The mAb and molecules detected by the mAb are shown in Table I. The fluorescein isothiocyanate (FITC)-conjugated AA4.1 mAb (rat IgG2b, κ), which recognizes pre-B cells, i.e., immature B lymphocytes, was also used in this study.

The single cell suspensions of bone marrow, thymus, spleen, lymph nodes and peripheral blood were respectively preincubated with unlabeled anti-Fcγ receptor mAb for 10 min at 4°C to avoid non-specific Fc-mediated binding of the labeled antibodies. The cells were stained with FITC-conjugated mAb and R-phycoerythrin (R-PE)-conjugated mAb simultaneously for 20 min at 4°C. In the staining of OX-40 antigen (CD134), the cells were stained with Biotin-conjugated OX-86 mAb (rat IgG1, κ) and streptavidin-phycoerythrin (SAv-PE) instead of the

Table I. Cell surface molecules detected and monoclonal antibodies used in this study, and the main immune cell populations expressing the molecules on their surfaces.

Molecules detected	Clone	Label [a]	Class	Cell [b]
Functional molecules				
CD16/32 (Fcγ III/II receptor)	2.4G2	Non	Rat IgG2b, κ	All
CD3 molecular complex	17A2	FITC	Rat IgG2b, κ	T
CD4	GK1.5	FITC, R-PE	Rat IgG2b, κ	T
CD8a (Ly-2)	53-6.7	FITC, R-PE	Rat IgG2a, κ	T
CD19	1D3	FITC, R-PE	Rat IgG2a, κ	B
CD45R/B220	RA3-6B2	FITC, R-PE	Rat IgG2a, κ	B
ICAM-1 (CD54)	3E2	R-PE	Hamster IgG, group 1, κ	T, B
Fas (CD95)	Jo2	R-PE	Hamster IgG, group 2, λ	T, B
NK-1.1 (NKR-P1, Ly-55)	PK136	R-PE	Mouse IgG2a, κ	NK
CXCR4 (CD184)	2B11/CXCR4	R-PE	Rat IgG2b	T, B
CCR5	C34-3448	R-PE	Rat IgG2c	T, M
Activation molecules				
THAM (CD26, DPP IV)	H194-112	FITC	Rat IgG2a, κ	T
CD28	37.51	R-PE	Hamster IgG, group 2, λ	T
CD40	3/23	R-PE	Rat IgG2a, κ	B
CD44H	TM-1	FITC	Hamster IgG, group 3, λ	T, B
CD70	FR70	R-PE	Rat IgG2b, κ	B
B7-1 (CD80)	16-10A1	R-PE	Hamster IgG, group 2, κ	B
B7-2 (CD86)	GL1	R-PE	Rat IgG2a, κ	T, B
OX-40 antigen (CD134)	OX-86	Biotin	Rat IgG1, κ	T
CTLA-4 (CD152)	UC10-4F10-11	R-PE	Hamster IgG, group 1, κ	T
CD30 ligand (CD153)	RM153	R-PE	Rat IgG2b, κ	T
CD40 ligand (gp39, CD154)	MR1	R-PE	Hamster IgG, group 3, κ	T

[a] FITC: Fluorescein isothiocyanate, R-PE: R-Phycoerythrin; [b] T: T cell, B: B cell, NK: Natural Killer cell, M: Macrophage or Monocyte.

above R-PE-conjugated mAb. After being washed, the stained samples were analysed by the EPICS XL flow cytometry system (Beckman Coulter, Fullerton, CA, USA).

Anti-SRBC PFC assay

To count the anti-SRBC antibody-producing cells, i.e., anti-SRBC PFC, the mice were sacrificed 4 days after SRBC-immunization. The single cell suspension was prepared from the spleen as described above. Incubation mixtures were prepared with a 490 μl RPMI 1640 medium supplemented with 10% FCS, a 10 μl spleen cell suspension, 50 μl of a 50% SRBC suspension, and 50 μl of a solution of the complement to enhance the hemolytic reaction. After the suspension was mixed gently in a water bath at 37°C for 5 min, it was plated in Cunningham chambers that were sealed with paraffin. After incubation for 1 h at 37°C in a 95% air/5% CO_2 incubator, PFC numbers were counted in triplicate for each mouse.

Statistical analysis

The percentages of immune cell populations, fluorescence intensities of the expression levels of cell surface molecules and the PFC numbers were presented as means ± standard errors. The statistical significance of the results for immune activity was evaluated using Student's *t*-test, after appropriate tests demonstrating that the percentages, intensities and numbers were normally distributed.

Results

In this study we examined the effects of continuous low-dose-rate irradiation on the immune system in whole body of wild-type mouse strains in comparison with acute high-dose-rate irradiation cases.

First, we analysed the changes in immune cell activities in the total body after irradiation. Figure 1 shows a cell population pattern that indicates a significant increase in the expression of CD8 molecules on the surface of thymic lymphocytes in the C57BL/6 mouse after continuous low-dose-rate irradiation at 1.2 mGy hour^{-1} for 3 weeks compared to an un-irradiated control mouse. The age of the mice was 5 weeks at the start of irradiation. CD4+ T cells and cell surface CD8 molecule expression on CD8+ T cells were also increased significantly in the spleen of the same mouse compared to the control mouse (Figure 2, upper and middle panels).

On the other hand, CD45R/B220+ CD40+ cells, i.e., CD40+ B cells, decreased in this same spleen (Figure 2, bottom panel). Figure 3 shows the time course of changes in immune cell activities in the

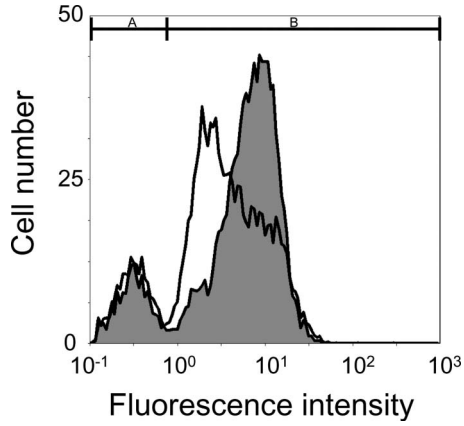

Figure 1. Increase of CD8 molecule expression on the surfaces of thymic lymphocytes in a C57BL/6 mouse after continuous γ-irradiation at a low dose-rate of 1.2 mGy hour^{-1} for 3 weeks from 5 weeks of age compared to an un-irradiated control mouse. The gates were set in the lymphocyte regions. (A) The region of CD8– thymic lymphocytes. (B) CD8+ thymic lymphocytes. Nearly 100% of the isotype controls were in the A regions. Open peaks without shadow: un-irradiated. Grey peaks with shadow: irradiated.

spleens of C57BL/6 mice continuously irradiated at a low dose-rate of 1.2 mGy hour^{-1} from 5 weeks of age compared to control mice. CD4+ T cells increased by 1.3-fold and then returned to the levels of the un-irradiated controls 35 days after the start of irradiation (Figure 3a). The cell surface CD8 molecule expression on CD8+ T cells increased by 1.3-fold on day 21, and returned to the levels of the un-irradiated controls on day 35 (Figure 3b). On the other hand, CD40+ B cells decreased 0.9-fold compared to the control level on day 7 and this level was maintained during the continuous irradiation (Figure 3c).

In the lymph nodes and peripheral blood in the low-dose-rate-irradiated mice, the same patterns as in the spleen cells were observed, although in the case of acute high-dose-rate irradiation at 1.6 Gy min^{-1} of X irradiation with 0.05–0.50 Gy, CD4+ T cell and CD8+ T cell populations showed tendencies to increase 2 days after irradiation; the increases, however, were not statistically significant (data not shown). Figure 4 shows the immune activities in the spleens of C57BL/6, BALB/c and C3H/He mice after continuous low-dose-rate γ irradiation at 1.2 mGy hour^{-1} for 3 weeks from 5 weeks of age. The same patterns in the changes of immune parameters seen in C57BL/6 mice were also observed in BALB/c and C3H/He mice. Furthermore, the results described above were also observed in other mouse strains used in this study: i.e., in DBA/1, DBA/2 and CBA mice (see Table II). No changes were observed in the numbers of cell populations expressing adhesion molecule ICAM-1 or chemokine receptors (CXCR4 or CCR5) on their surfaces in the continuously low-dose-rate-irradiated mice compared to

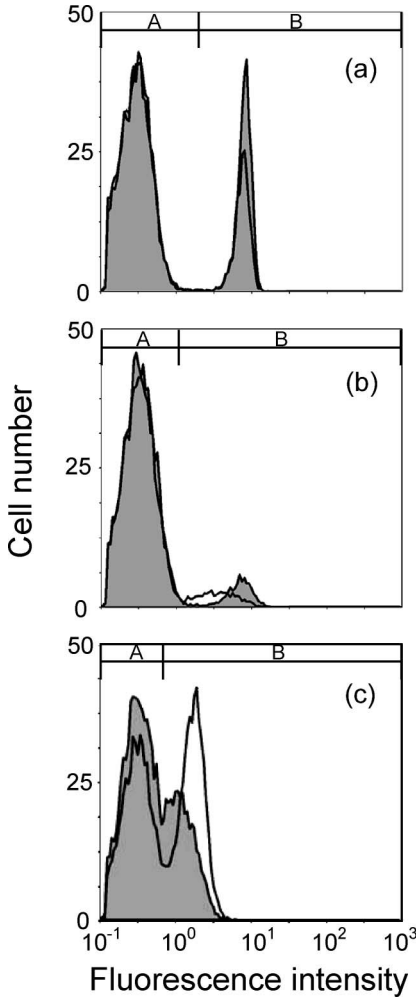

Figure 2. Changes in immune cell populations in the spleen of a C57BL/6 mouse after continuous γ irradiation at a low dose-rate of 1.2 mGy hour^{-1} for 3 weeks from 5 weeks of age compared to an un-irradiated control mouse. The gates were set in the lymphocyte regions. *Panel a*: CD4+ T cells. (A) The region of CD4− lymphocytes. (B) CD4+ lymphocytes, i.e. CD4+ T cells. *Panel b*: CD8+ T cells. (A) CD8− lymphocytes. (B) CD8+ lymphocytes, i.e. CD8+ T cells. *Panel c*: CD40+ B cells. (A) CD40− lymphocytes. (B) CD40+ lymphocytes, i.e. CD40+ B cells. Nearly 100% of the isotype controls were in the A regions. Open peaks without shadow: un-irradiated. Grey peaks with shadow: irradiated.

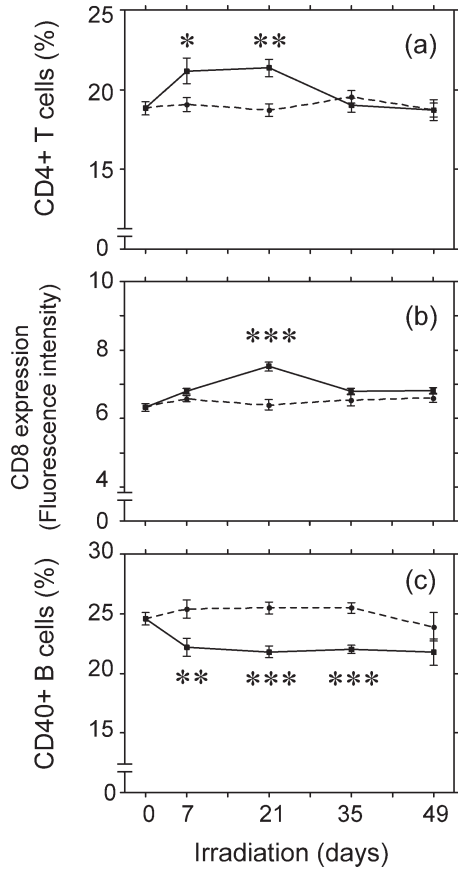

Figure 3. Changes in immune cell populations and in expression of cell surface molecules on immune cells in the spleens of C57BL/6 mice during continuous low-dose-rate γ irradiation at 1.2 mGy hour^{-1} from 5 weeks of age compared to un-irradiated control mice. Solid curves: irradiated. Dotted curves: un-irradiated. *Panel a*: CD4+ T cells. *Panel b*: CD8 molecule expressions. *Panel c*: CD40+ B cells. $n = 12$ per point. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.0001$ compared to controls.

control mice (data not shown). In the acute high-dose-rate irradiation cases, no significant activation of the immune system was observed after irradiation (data not shown).

Secondly, we analysed the changes in immune cell activities in the whole body after SRBC-immunization, after the mice were acutely X-irradiated at a high dose-rate of 1.6 Gy min^{-1} or while continuously low-dose-rate γ-irradiated at 1.2 mGy hour^{-1}. Populations of CD4+ T cells and CD40+ B cells increased and reached a plateau 21 days after the start of continuous low-dose-rate irradiation. Thereafter, the elevated levels were maintained during the continuous low-dose-rate irradiation (Figures 5a–b).

Splenic PFC increased with time in response to SRBC after the start of the continuous low-dose-rate irradiation, reaching a level 2.4 times the level of the unirradiated mice (Figure 5c). The pattern of the time course of this increase was very similar to those for CD4+ T cell and CD40+ B cell increases. The results here were more clearly shown than in the previous report (Ina et al. 2005), although the mouse colony used (Charles River colony vs. Clea colony) was different, again confirming the results. In the lymph nodes and peripheral blood, the same patterns as seen in these results were observed (data not shown). In the case of acute high-dose-rate irradiation, no significant activation of the immune system was observed after SRBC-immunization (data not shown).

Lastly, we measured the CD3− CD4+ lymphocytes after acute high-dose-rate or continuous low-dose-rate irradiations respectively, when the cell numbers were at their peak (Figure 6).

The fact that there were no changes in numbers of cell populations expressing NK cell marker

NK-1.1 or dendritic cell marker CD11c on their surfaces (Janeway Jr et al. 2001) in the mice irradiated at acutely high-dose-rate or continuously low-dose-rate indicated that the CD3− CD4+ lymphocytes were CD3− CD4+ T cells (data not shown). In the C57BL/6 mice acutely high-dose-rate-irradiated at 1.6 Gy min^{-1}, the CD3− CD4+ T cells increased in a dose-dependent fashion. We regard the presence of this cell population as representing the formation of abnormal immune cells. On the other hand, in the C57BL/6 mice continuously low-dose-rate-irradiated at 1.2 mGy $hour^{-1}$, the abnormal T cells were absent throughout the dose range examined.

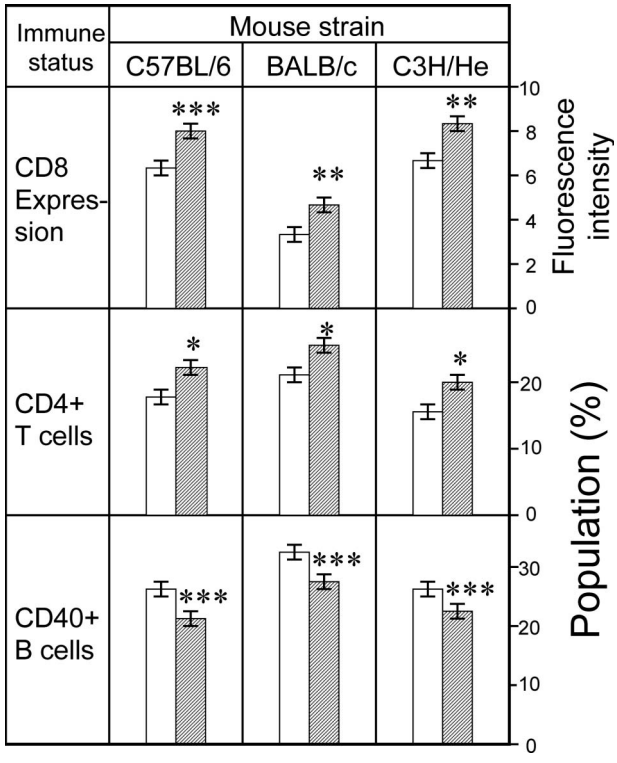

Figure 4. Changes in immune cell populations and in expression of cell surface molecules on immune cells in the spleens of C57BL/6, BALB/c and C3H/He mice after continuous low-dose-rate γ irradiation at 1.2 mGy $hour^{-1}$ for 3 weeks from 5 weeks of age compared to un-irradiated control mice. Open bars: un-irradiated. Hatched bars: irradiated. $n = 12$ per point. $^{*}p < 0.01$, $^{**}p < 0.001$, $^{***}p < 0.0001$ compared to controls.

Furthermore, as to the total cell numbers and the numbers of subpopulation cells expressing adhesion molecules or chemokine receptors, there were no significant changes in the bone marrow, thymus, spleen, lymph nodes and peripheral blood after chronic low-dose-rate irradiation alone. There were also no injuries in the intestine and no radiation-induced lymphomas in the low-dose-rate-irradiated mice (data not shown). Tendencies, however, even though statistically not significant (data not shown) were recognized for increases in various activation molecules in the low-dose-rate-irradiated mice with SRBC, as were tendencies for increases in adhesion molecules and chemokine receptors, plus tendencies for decreases in cell number in the bone marrow, thymus, spleen, lymph nodes and peripheral blood in the acutely high-dose-rate-irradiated mice in the dose range 0.50 – 1.0 Gy.

In this study we observed no significant difference between the unirradiated controls and the low-dose-rate-irradiated mice, whether in the percentages of thymocytes stained by propidium iodide, trypan blue and erythrosin B, or in the expressions of phosphatidylserine on the thymocytes. Furthermore, there was no increase in expression of the apoptosis-regulating molecule Fas (CD54) on the thymocytes, splenocytes, lymph node cells or on the peripheral blood cells in the low-dose-rate-irradiated mice, although statistically non-significant tendencies for increases in the numbers of stained thymocytes and Fas molecule expressions were observed in the mice acutely high-dose-rate X-irradiated at 1.6 Gy min^{-1} with 0.50 – 1.0 Gy (data not shown).

Table II. Changes in immunological activities in various wild-type mouse strains after chronic low-dose-rate γ irradiation.

	Relative immunological parameters [a]					
	$CD4^+$ T cells [b]		$CD8^+$ T cells [c]		$CD40^+$ B cells [b]	
Mouse strain	Control	Irradiated	Control	Irradiated	Control	Irradiated
C57BL/6	1.0	1.3	1.0	1.3	1.0	0.88
BALB/c	1.0	1.3	1.0	1.2	1.0	0.89
C3H/He	1.0	1.2	1.0	1.3	1.0	0.90
DBA/1	1.0	1.2	1.0	1.2	1.0	0.91
DBA/2	1.0	1.2	1.0	1.2	1.0	0.90
CBA	1.0	1.2	1.0	1.2	1.0	0.90

[a] Measured in splenic lymphocytes of 12 mice in each group continuously γ-irradiated at a low dose-rate of 1.2 mGy $hour^{-1}$ for 3 weeks beginning at 5 weeks of age and sacrificed at 8 weeks of age; [b] Percentage of Population; [c] Fluorescence intensity of CD8 molecules expressed on the cell surfaces.

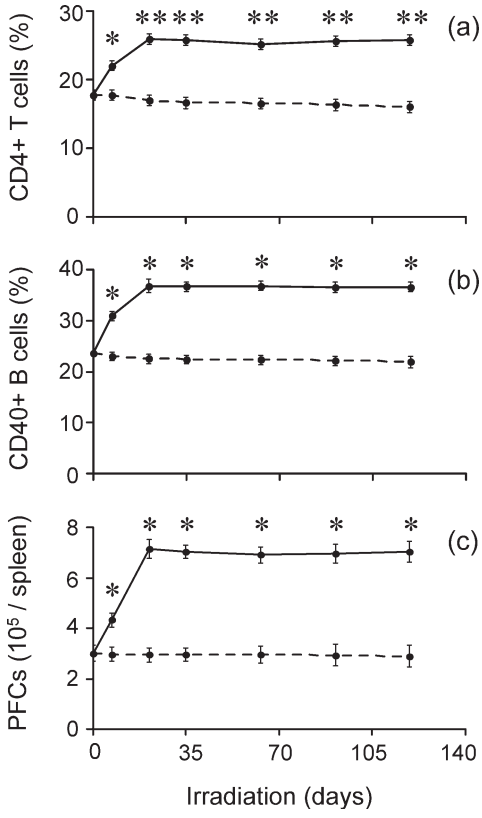

Figure 5. Activation of immune cell populations in the spleens of C57BL/6 mice challenged intraperitoneally by SRBC plotted as a function of time by continuous low-dose-rate γ irradiation at 1.2 mGy hour^{-1} from 5 weeks of age compared to un-irradiated control mice. Solid lines: irradiated. Dotted lines: un-irradiated. *Panel a*: CD4+ T cells (*$p < 0.01$ and **$p < 0.0001$ to the control). *Panel b*: CD40+ B cells, i.e. activated B cells (*$p < 0.0001$). *Panel c*: PFC, i.e. anti-SRBC antibody-producing cells (*$p < 0.0001$). $n = 12$ per point.

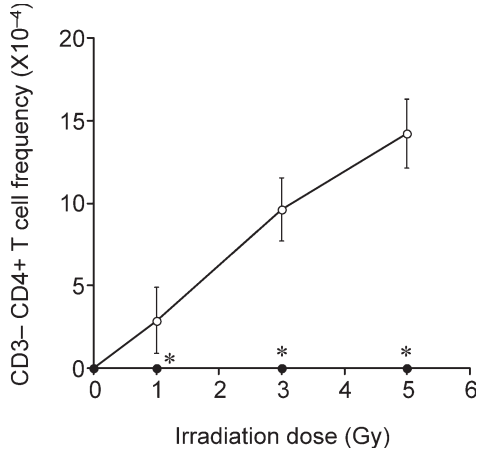

Figure 6. Effects of dose-rate on the formation of abnormal immune cells, CD3– CD4+ lymphocytes, i.e., CD3– CD4+ T cells in the spleens of C57BL/6 mice. Open circles: 1.6 Gy min^{-1}, acute. Closed circles: 1.2 mGy hour^{-1}, chronic. $n = 12$ per point. *$p < 0.0001$ to the acutely high-dose-rate-irradiated mice.

Discussion

In this study, we found a significant activation of the immune system in the whole body of wild-type mouse strains by chronic low-dose-rate irradiation both before and after the immunization with antigens.

CD4+ T cells and CD8 molecules on the surfaces of CD8+ T cells increased after the start of continuous low-dose-rate γ irradiation at 1.2 mGy hour^{-1}, reached a plateau, and then returned to the levels of unirradiated controls. On the other hand, CD40+ B cells decreased after the start of continuous low-dose-rate irradiation and reached a low level, after which the decreased levels were maintained during continuous low-dose-rate irradiation. CD4 molecules are the counterparts of class II major histocompatibility complex (MHC) molecules, and CD8 molecules the counterparts of class I MHC molecules in antigen-presenting steps in the immune system. CD40+ B cells are one of the major cell populations in the progression of inflammation and autoimmune diseases when the hosts are free of pathologic antigens (Janeway Jr et al. 2001, Mohan et al. 1995). No changes were found in the numbers of cell populations expressing adhesion molecules or chemokine receptors on their surfaces in the continuously low-dose-rate-irradiated mice, again indicating the absence of inflammation and autoimmune diseases in the mice. Without antigens, under SPF conditions, as in this study, continuously excessive activation of the immune system causes inflammation and autoimmune diseases (Janeway Jr et al. 2001). It would therefore seem that a moderate activation of the immune system without causing excessive immune activation should be able to be achieved by chronic low-dose-rate irradiation.

Under a specific antigen condition, i.e., with injection of SRBC, CD4+ T cells and CD40+ B cells increased and reached a plateau after the start of continuous low-dose-rate irradiation. Thereafter, the elevated levels were maintained during continuous low-dose-rate irradiation. The patterns of increases of PFC were very similar to those for CD4+ T cell and CD40+ B cell increases. CD40+ B cells are one of the major cell populations in activated immune status when specific antigens exist in the body (Janeway Jr et al. 2001). Therefore, with chronic low-dose-rate irradiation, the immune system seems to be activated effectively in concert with increases in CD4+ T cells and CD40+ B cells, leading to augmentation of PFC, i.e., the antibody-producing cells against the antigens. Furthermore, tendencies were observed for increases in the numbers of cell populations expressing various activation molecules on their surfaces in the chronically

low-dose-rate-irradiated mice challenged with SRBC, again indicating an augmentation of immune activity.

In addition, we analysed the emergence of abnormal immune cells after acute high-dose-rate or continuous low-dose-rate irradiations. In the mice acutely high-dose-rate X-irradiated at 1.6 Gy min^{-1}, the abnormal immune cells increased dose-dependently. On the other hand, in the mice continuously low-dose-rate γ-irradiated at 1.2 mGy $hour^{-1}$, no abnormal immune cells were detected at all irradiation doses, indicating an inefficiency of chronic low-dose-rate irradiation for the induction of abnormal immune cells.

In previous reports (Ina & Sakai 2004, 2005, Ina et al. 2005), we showed a remarkable prolongation of life span, suppression of tumour induction even with high-dose radiation at a high dose-rate, and suppression of severe multiple disease and severe autoimmune disease, together with activation of the immune system by chronic low-dose-rate γ irradiation at the same dose-rate as in this study and also at a lower dose-rate of 0.35 mGy $hour^{-1}$, indicating curative effects of low-dose-rate irradiation for these diseases. Furthermore, Ina et al. observed no tumour metastases produced by acute high-dose-rate irradiation with 4 repeats of 1.8 Gy, when combined with a continuous low-dose-rate irradiation, while tumour metastases were found in the lymph nodes, liver, kidneys and lungs in the acutely high-dose-rate-irradiated mice without low-dose-rate treatment (unpublished data). In the present study, we showed a significant augmentation of immune activity even with specific antigens, together with the absence of abnormal immune cells. Even so, from the standpoint of cytokines and other soluble mediator activities and signal transduction molecules, a more intensive study may be required using different dose-rates in various mouse strains, other animals and humans. Chronic low-dose-rate radiation may have stimulative effects that activate the immunological network in the whole body and suppress various diseases including tumours and infectious diseases.

In conclusion, we showed total body immunological network activation without causing abnormal immune cells by chronic low-dose-rate irradiation, suggesting that such radiation is useful in the activation of the immune system in the whole body.

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