



Dietary factors and cancer mortality among atomic-bomb survivors[☆]

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Abstract

Dietary factors such as fruit and vegetables are thought to reduce the risk of cancer incidence and mortality. We investigated the effect of a diet rich in fruit and vegetables against the long-term effects of radiation exposure on the risk of cancer. A cohort of 36,228 atomic-bomb survivors of Hiroshima and Nagasaki, for whom radiation dose estimates were currently available, had their diet assessed in 1980. They were followed for a period of 20 years for cancer mortality. The joint-effect of fruit and vegetables intake and radiation exposure on risk of cancer death was examined, in additive (sum of effects of diet alone and radiation alone) and multiplicative (product of effects of diet alone and radiation alone) models. In the additive model, a daily intake of fruit and vegetables significantly reduced the risk of cancer deaths by 13%, compared to an intake of once or less per week. Radiation exposure of 1 Sievert (Sv) increased significantly the risk of cancer death by 48–49%. The additive joint-effects showed a lower risk of cancer among those exposed to 1 Sv who had a diet rich in vegetables (49% – 13% = 36%) or fruit (48% – 13% = 35%). The multiplicative model gave similar results. The cancer risk reduction by vegetables in exposed persons went from 52% (effect of radiation alone) to 32% (product of effect of vegetables and radiation), and cancer risk reduction by fruit was 52% (radiation alone) to 34% (product of effect of fruit and radiation). There was no significant evidence to reject either the additive or the multiplicative model. A daily intake of fruit and vegetables was beneficial to the persons exposed to radiation in reducing their risks of cancer death.

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1. Introduction

Ionising radiation exposure is a well-established carcinogenic risk factor. In a cohort of Japanese

atomic-bomb survivors who received a broad range of doses (mean 0.1 Sv), radiation accounts for 5% of cancer deaths [1]. A diet rich in fruit and vegetables is thought to reduce the cancer risks, especially digestive cancers [2]. This protective effect was indeed observed among the cited cohort of A-bomb survivors, where the risks of all solid cancer deaths were significantly reduced by 8% for those with a high vegetables consumption, and by 12% for those with a high fruit intake [3,4]. Fruit and vegetables

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are a rich source of antioxidants and numerous other compounds, and have shown anti-carcinogenic effects in epidemiological studies [5,6]. However, clinical trials on dietary antioxidant supplementation did not report convincing protective effects against cancer incidence or mortality [7–9]. Yet, the protective effects might also be explained by other micronutrients such as isothiocyanates, allium compounds, isoflavones, protease inhibitors, saponins, and dietary fiber [10].

The potential of dietary antioxidants to reduce the cellular damage induced by ionising radiation has been recently reviewed [11]. Numerous laboratory studies have described the protective effects of vitamins and other dietary factors supplemented *before* radiation exposure, examining their radio-protective effects. However, few have reported their effects *after* irradiation. Dietary factors such as flavorings (anisaldehyde, cinamaldehyde, coumarin, and vanillin) have shown to be effective even when applied after X-irradiation to reduce radiation-induced mutations [12], and chromosome aberrations [13]. These compounds have also been effective in mice [14]. Ascorbic acid and beta-carotene have reduced the radiation effects on the frequency of mutations and morphological transformations in cultured mammalian cells [15] and in vivo studies [16,17], even when added after irradiation.

As compared to laboratory experiments, human studies are very limited. Clinical trials involving persons exposed to ionising radiation during medical treatment or for professional reasons have evaluated the early effects of a supplementation of antioxidants on chronic radiation damage [11]. The findings showed a regression of the symptoms at high doses of combined antioxidants. However, little is known of the late protection effect of antioxidants on carcinogenesis, when the cellular damage was initiated by earlier radiation exposure. In animal studies, antioxidants such as selenium and Vitamin D have been suggested to prevent cancer promotion, but the effect of such antioxidants on cancer progression in humans is still unknown [2].

We have previously reported that intake of fruit and vegetables reduced significantly the risk of death from digestive and respiratory cancers [3]. In the present study, diet was assessed 35 years after atomic-bomb radiation exposure, among A-bomb survivors of Hiroshima and Nagasaki. The aim of the present study was to analyse the effect of a daily consumption of

fruit and vegetables on the long-term effects of radiation on cancer risk.

2. Study subjects and methods

2.1. Study population

The life span study (LSS) is a longitudinal study of 120,321 persons who have been exposed to the atomic bombings in Hiroshima and Nagasaki, and non-exposed controls. The cohort follow-up was initiated in 1950 by the Atomic Bomb Casualty Commission (ABCC), followed by the Radiation Effects Research Foundation [18].

The part of the LSS cohort used in the present study included 36,228 individuals who were alive as of September 1, 1978, and responded to a lifestyle mail questionnaire in 1980, and for whom radiation dose estimates were currently available [19]. The prevalent cases of cancer at baseline ($n = 1159$), and the death occurring within the first 2 years of follow-up ($n = 251$) were excluded from the analyses. The total study population consisted of 34,818 persons.

Among them, 12,839 persons had dose estimates of less than 0.005 Sv (36.9% of the population). The mean dose for the remaining 21,979 cohort participants with dose estimates higher than 0.005 Sv was 0.20 Sv. The distribution of the participants by radiation dose and city is shown in Table 1.

2.2. Dosimetry/radiation dose assessment

Individual doses for survivors were estimated based on detailed models of the dose–distance relationship and of how doses are affected by shielding [18]. The dosimetry system DS86 was used, giving individual estimates of gamma ray and neutron exposures [20]. The weighted organ dose (in Sievert or Sv) was calcu-

Table 1
Number of subjects by radiation dose and city

City	Total	DS86 weighted colon dose (Sv)			
		0 (≤ 0.005)	0.005–0.5	0.5–1.0	>1.0
Hiroshima	26,451	9,815	15,081	950	605
Nagasaki	8,367	3,024	4,382	600	361
Total	34,818	12,839	19,463	1,550	966

lated as the sum of the gamma ray dose plus 10 times the neutron dose. The factor 10 represented the relative biological effectiveness (RBE) of neutrons, because neutrons were suspected to have a greater biological effect than gamma rays [18]. Adjusted doses were used to reduce bias in risk estimates.

2.3. Dietary assessment

A lifestyle questionnaire was sent to the LSS participants between June and November 1979, and the mail survey was completed on January 1, 1980 for men, and February 1, 1981 for women [19]. The questionnaire included items on past medical history, marital status, anthropometrical information, smoking and drinking habits, a 22-item food frequency questionnaire (FFQ), occupation, and education.

In the present study, we used the answers to two dietary factors: green–yellow vegetables and fruit. The average consumption within the previous year was categorized into “never and once a week or less”, “twice to four times a week”, and “daily or almost daily”. The validity of the FFQ has been previously reported, showing moderate correlation with the 24-h diary used as reference method [21].

2.4. Follow-up of mortality

The follow-up carried out regular checks on the vital status of the LSS participants, through the Japanese family registration system (Koseki). The Koseki provides complete coverage for all LSS members residing in Japan [18]. Copies of death certificates are regularly obtained by RERF for all deceased LSS participants, and trained coders enter the appropriate codes into the database. Causes of death were coded according to the International Classification of Diseases, ninth and tenth revisions [22]. The follow-up was started on January 1, 1980 for men and February 1, 1981 for women, and continued until the date of death or December 31, 1999, whichever came first.

We previously reported that consumption of green–yellow vegetables and fruit had a significant protective effect on total cancer, stomach, liver and lung cancer mortality [3]. Therefore, the current analyses will focus on total solid cancer and the three significant sites.

2.5. Statistical analysis

To examine the joint effects of vegetables or fruit consumption and radiation exposure on cancer risk, the following two hazard risk models were applied using the dummy variables, m for moderate, and h for high consumption of vegetables or fruit:

- Additive model:

$$\text{Relative risk} = e^{\gamma Z} (1 + \beta_1 m + \beta_2 h + \beta_3 d)$$

- Multiplicative model:

$$\text{Relative risk} = e^{\gamma Z} (1 + \beta_1 m + \beta_2 h)(1 + \beta_3 d)$$

where Z represents other cancer risk factors: city (Hiroshima, Nagasaki); sex; age at the start of follow-up; smoking habits (never, current, past); drinking habits (never, current, past); and education level (low, middle, high); and where $m = 1$ for moderate intake group of vegetables or fruit, and 0 for low and high intake groups; $h = 1$ for high intake group of vegetables or fruit, and 0 for low and moderate intake groups; d is the radiation dose in Sievert.

For each model, the test for data fitting was measured in comparing the deviances between the additive and the multiplicative models.

Relative risks were estimated using the PEANUTS procedure of the EPICURE software [23].

3. Results

The total study population included 13,309 men, and 21,509 women. The mean age was 55 years (range = 34–98 years).

During the follow-up period of 20 years, 2628 cancer deaths were identified, including 522 deaths due to stomach cancer, 477 liver cancer deaths, and 458 lung cancer deaths.

Table 2 shows the percentage consumption of green–yellow vegetables and fruit, according to the radiation dose exposure. Most of the participants had vegetables twice to four times a week, and fruit everyday. The distribution was similar across the radiation exposure categories.

Table 3 presents the percentage change in cancer risk, according to vegetables and fruit intake, and to

Table 2
Intake distribution (in percentage) by radiation dose

Dose (Sv)	Green–yellow vegetables			Fruit		
	≤1/week	2–4/week	Daily	≤1/week	2–4/week	Daily
0 (≤0.005)	31.7	43.8	24.5	23.5	31.0	45.5
0.005–0.5	32.0	43.1	24.9	24.3	31.5	44.2
0.5–1.0	32.6	44.3	23.1	25.0	31.7	43.3
>1.0	35.0	43.5	21.5	25.2	32.9	41.9

radiation exposure, in two different interaction models. The first model, the additive model, implies that the combined effects of diet and radiation on the cancer risk is the sum of the effect of diet in non-exposed persons, and the effects of radiation in subjects with low intake of the dietary factor. The second model, the multiplicative model, refers to the combined effects of diet and radiation that is the product of their effects alone; in other words, the higher dose a person received, the more effective the diet.

For solid cancer, in the additive model, the persons with a daily consumption of vegetables or fruit, without any exposure to radiation, had a 13% decreased risk of cancer. Those exposed at 1 Sv, and with a low consumption of vegetables or fruit, had a 48–49% increased risk of cancer. The joint-effects showed consequently a lower risk of cancer among those exposed to 1 Sv who had a diet high in vegetables and fruit, as compared to those with a low diet (vegetables: risk of 36% instead of 49%). The multiplicative model gave similar results. The effect of a diet rich in vegetables or fruits decreased the risk of cancer due to radiation from 52 to 32–34%.

As for specific cancer sites, the effect of a daily intake of vegetables or fruit was through a reduction of cancer risk. The results are consistent with previous findings in the same cohort, where we showed that fruit decreased significantly the risk of stomach cancer, and vegetables the risk of liver cancer [3]. Not surprisingly, the risk of cancers increased significantly with radiation exposure; and the joint-effects of diet and radiation showed a lower risk of cancer as compared to the effects of radiation only, suggesting that a diet rich in vegetables and fruit benefits persons exposed to atomic radiation. We also checked the multiplicative joint-effects for the specific cancer sites. The effects were similar to the additive effects, consequently the results are not shown.

For the additive and multiplicative models, the test for lack of fit showed a *P*-value greater than 0.5. This means that there is no significant evidence for rejecting either the additive or the multiplicative model.

4. Discussion

A diet rich in green–yellow vegetables and fruit reduced the risk of cancer among those exposed to ionising radiation. However, we were not able to rule out that there was any additive or multiplicative effect of diet on the radiation effect. The main reason was that the data did not have enough power. Most of the study subjects included in the LSS cohort received a very low radiation dose. Those exposed to 1 Sv or more represented less than 3% of the population (see Table 1). The radiation effect in this cohort has a low power. It explains only 5% of the cancer risks because most of the survivors received a low radiation dose [1]. Additionally, the effects of diet on cancer risk in human beings are generally low. Thus, in the present population, it was difficult to show any risk difference modification at a significant level. Nevertheless, despite the low power of the data, the findings cannot exclude a post-irradiation, long-term protective effect of dietary factors on cancer risk.

Studies on effects of diet on cancer incidence or mortality in radiation-exposed persons are limited [11]. One study reported that a high intake of fresh fruit and vegetables was associated with an increased immune protection in young children exposed to the Chernobyl accident. The protective effects were observed 3–7 years after the radiation exposure [24].

Fruit and vegetables are a rich source of antioxidants. Several clinical trials on the effects of antioxidant nutrients on chronic radiation damage among therapeutically irradiated persons have been pub-

Table 3
Percentage of change in risk of cancer, in additive and multiplicative models, according to the dietary factor

	Additive effects of diet and radiation dose					
	Green–yellow vegetables			Fruit		
	% Change	95% CI	P-value	% Change	95% CI	P-value
All solid cancers						
Effect of a high intake of dietary factor in non-exposed to radiation subjects	–13	(–22 –03)	0.0073	–13	(–22 –04)	0.0047
Effect of radiation exposure at 1 Sv with low intake of dietary factor	+49	(+32 +66)	0.0001	+48	(+31 +65)	0.0001
Effect of a high intake of dietary factor in radiation exposed subjects	+36			+35		
Stomach cancer						
Effect of a high intake of dietary factor in non-exposed to radiation subjects	–13	(–34 +08)	0.2190	–19	(–38 –01)	0.0394
Effect of radiation exposure at 1 Sv with low intake of dietary factor	+44	(+06 +83)	0.0229	+42	(+05 +79)	0.0240
Effect of a high intake of dietary factor in radiation exposed subjects	+31			+23		
Lung cancer						
Effect of a high intake of dietary factor in non-exposed to radiation subjects	–08	(–33 +17)	>0.5	–17	(–37 +04)	0.1070
Effect of radiation exposure at 1 Sv with low intake of dietary factor	+66	(+20 +112)	0.0051	+61	(+18 +104)	0.0051
Effect of a high intake of dietary factor in radiation exposed subjects	+58			+44		
Liver cancer						
Effect of a high intake of dietary factor in non-exposed to radiation subjects	–28	(–47 –09)	0.0039	–02	(–25 +21)	>0.5
Effect of radiation exposure at 1 Sv with low intake of dietary factor	+47	(+10 +84)	0.0121	+54	(+12 +96)	0.0108
Effect of a high intake of dietary factor in radiation exposed subjects	+19			+52		
Multiplicative effect of diet and radiation dose						
All solid cancers						
Effect of a high intake of dietary factor in non-exposed to radiation subjects	–13	(–22 –04)	0.0056	–12	(–21 –04)	0.0051
Effect of radiation exposure at 1 Sv with low intake of dietary factor	+52	(+32 +72)	0.0001	+52	(+34 +70)	0.0001
Effect of a high intake of dietary factor in radiation exposed subjects	+32			+34		

lished. Oral supplementation of a combination of pentoxifylline (800 mg per day) and Vitamin E (1000 IU per day), for at least 6 months, reversed cutaneous radiation syndrome in patients who had radiotherapy for head, neck or breast cancers 0.5–30 years before [25,26]. The same combination of pentoxifylline and Vitamin E reduced fibroatrophic uterine lesions in women who were irradiated in the pelvic area, 25

years ago [27]. Similarly, supplementation of Vitamin E (1200 IU per day) and Vitamin C (1.5 g per day) improved the symptomatology of chronic radiation proctitis following radiotherapy for prostatic and gynaecological cancers [28].

At the biological level, two clinical trials on the effects of a supplementation of nutrients have been reported in persons exposed to radiation from the Cher-

nobyl accident. Ben-Amotz et al. [29] showed that beta-carotene supplementation reduced the blood level of oxidation. Emerit et al. [30] also demonstrated that supplementation of antioxidants from Ginkgo biloba leaves (containing flavonoids and terpenoids) for 2 months reduced the clastogenic activity of the plasma in Chernobyl liquidators. Studies on the existence of persistent clastogenic factors or genomic instability among irradiated persons have reported conflicting results. Some demonstrated the persistence of clastogenic factors [31,32], others showed no enhanced genomic instability [33–35].

Mechanisms of cancer protection by dietary nutrients that can modulate radiation responses are far from clear. Antioxidants and other dietary substances may interfere with DNA damage. However, it is unclear whether their anti-mutagenic properties have any anti-carcinogenic effect [11].

Although our study was performed in a general setting, the findings go into the same direction as the results of clinical trials, even if they are not as strong as trials. The main reason has been discussed earlier; several other arguments are discussed now.

First, the daily dietary antioxidant intakes of the usual diet were at a much lower dose than supplementation intakes of clinical trials. It has been estimated in Japan that, in 1980, the daily per capita intake of Vitamin E was 12 IU (1000–1200 IU in trials), and Vitamin C was 123 mg (1500 mg in trials) [36,37]. It is possible that the usual diet amount was too low to show any strong joint-effect with past radiation exposure. It should also be noted that information on personal vitamin supplementation was not available in the present cohort. Moreover, while our findings suggested a protective role for fruit and vegetables, we cannot assume whether antioxidants and/or micronutrients were the protective factors.

Second, diet was assessed 35 years after ionising radiation exposure. Previous clinical studies reported an effect after a shorter time lag. The cancer promotion might have already occurred, while it has been hypothesised that antioxidant vitamins were more effective at the early stage of the carcinogenesis, like the initiation or promotion stages [2,38,39]. It would have been more appropriate to measure the fruit and vegetables intake just after the radiation exposure, i.e. in August 1945. According to the National Nutrition Survey, during the war and post-war years, fruit and vegetables

intake per capita per day in Japan was very low (fruit, 16 g per day; vegetables, 76 g per day in 1949) [40]. This suggests that during the cancer initiation—several decades before the onset—fruit and vegetables compounds were at a low level and might have been ineffective.

Finally, we cannot rule out dietary measurement errors. Diet information is based on the response to a single food frequency questionnaire. During the follow-up period of 16 years, fruit and vegetables consumption among the cohort participants has likely changed. In the general population, within the last two decades, fruit consumption has decreased (1980, 155 g per day; 1998, 116 g per day), while vegetables intake has increased (1980, 251 g per day; 1998, 261 g per day) [36]. Moreover, measurement errors might have been due to the absence of information on the fruit and green–yellow vegetables types, or on the portion size, preventing us from estimating the nutrient intake and consequently performing more accurate analyses.

In conclusion, in this large cohort of atomic-bomb survivors, a daily intake of fruit and vegetables benefited those exposed to radiation in reducing their risk of cancer, at least to the same extent as those not exposed to radiation.

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References

- [1] D.L. Preston, Y. Shimizu, D.A. Pierce, A. Suyama, K. Mabuchi, Studies of mortality of atomic bomb survivors, report 13: solid cancer and non-cancer disease mortality: 1950–1997, *Radiat. Res.* 160 (2003) 381–407.
- [2] World Cancer Research Fund and American Institute for Cancer Research, Food, nutrition and the prevention of cancer:

- a global perspective, American Institute for Cancer Research, Washington, 1997.
- [3] C. Sauvaget, J. Nagano, M. Hayashi, E. Spencer, Y. Shimizu, N. Allen, Vegetables and fruit intake and cancer mortality in the Hiroshima/Nagasaki life span study, *Br. J. Cancer* 88 (2003) 689–694.
- [4] J. Nagano, S. Kono, D.L. Preston, H. Moriwaki, G.B. Sharp, K. Koyama, K. Mabuchi, Bladder-cancer incidence in relation to vegetable and fruit consumption: a prospective study of atomic-bomb survivors, *Int. J. Cancer* 86 (2000) 132–138.
- [5] G. Block, B. Patterson, A. Subar, Fruit, vegetables, and cancer prevention: a review of the epidemiological evidence, *Nutr. Cancer* 18 (1992) 1–29.
- [6] M.J. Hill, Nutrition and human cancer, *Ann. N.Y. Acad. Sci.* 833 (1997) 68–78.
- [7] I.M. Lee, N.R. Cook, J.E. Manson, J.E. Buring, C.H. Hennekens, Beta-carotene supplementation and incidence of cancer and cardiovascular disease: the women's health study, *J. Natl. Cancer Inst.* 91 (1999) 2102–2106.
- [8] W.J. Blot, J.Y. Li, P.R. Taylor, W. Guo, S. Dawsey, G.Q. Wang, C.S. Yang, S.F. Zheng, M. Gail, G.Y. Li, et al., Nutrition intervention trials in Linxian, China: supplementation with specific vitamin/mineral combinations, cancer incidence, and disease-specific mortality in the general population, *J. Natl. Cancer Inst.* 85 (1993) 1483–1492.
- [9] G.S. Omenn, G.E. Goodman, M.D. Thornquist, J. Balmes, M.R. Cullen, A. Glass, J.P. Keogh, F.L. Meyskens, B. Valanis, J.H. Williams, S. Barnhart, S. Hammar, Effects of a combination of beta carotene and Vitamin A on lung cancer and cardiovascular disease, *N. Engl. J. Med.* 334 (1996) 1150–1155.
- [10] K.A. Steinmetz, J.D. Potter, Vegetables, fruit, and cancer. I. Epidemiology, *Cancer Causes Control* 2 (1991) 325–357.
- [11] J.F. Weiss, M.R. Landauer, Protection against ionizing radiation by antioxidant nutrients and phytochemicals, *Toxicology* 189 (2003) 1–20.
- [12] H. Imanishi, Y.F. Sasaki, K. Matsumoto, M. Watanabe, T. Ohta, Y. Shirasu, K. Tutikawa, Suppression of 6-TG-resistant mutations in V79 cells and recessive spot formations in mice by vanillin, *Mutat. Res.* 243 (1990) 151–158.
- [13] Y.F. Sasaki, H. Imanishi, M. Watanabe, T. Ohta, Y. Shirasu, Suppressing effect of antimutagenic flavorings on chromosome aberrations induced by UV-light or X-rays in cultured Chinese hamster cells, *Mutat. Res.* 229 (1990) 1–10.
- [14] Y.F. Sasaki, T. Ohta, H. Imanishi, M. Watanabe, K. Matsumoto, T. Kato, Y. Shirasu, Suppressing effects of vanillin, cinnamaldehyde, and anisaldehyde on chromosome aberrations induced by X-rays in mice, *Mutat. Res.* 243 (1990) 299–302.
- [15] S. Koyama, S. Kodama, K. Suzuki, T. Matsumoto, T. Miyazaki, M. Watanabe, Radiation-induced long-lived radicals which cause mutation and transformation, *Mutat. Res.* 421 (1998) 45–54.
- [16] A. Ben-Amotz, B. Rachmilevich, S. Greenberg, M. Sela, Z. Weshler, Natural beta-carotene and whole body irradiation in rats, *Radiat. Environ. Biophys.* 35 (1996) 285–288.
- [17] R.S. Harapanhalli, V. Yaghmai, D. Giuliani, R.W. Howell, D.V. Rao, Antioxidant effects of Vitamin C in mice following X-irradiation, *Res. Commun. Mol. Pathol. Pharmacol.* 94 (1996) 271–287.
- [18] D.A. Pierce, Y. Shimizu, D.L. Preston, M. Vaeth, K. Mabuchi, Studies of the mortality of atomic bomb survivors, report 12, Part I. cancer: 1950–1990, *Radiat. Res.* 146 (1996) 1–27.
- [19] Mail Questionnaire Survey for Epidemiologic Data on the Life-span Study Extended Sample, Radiation Effects Research Foundation, Hiroshima, 1978.
- [20] W.C. Roesh, Final Report on the Reassessment of Atomic-Bomb Radiation Dosimetry in Hiroshima and Nagasaki, Radiation Effects Research Foundation, Hiroshima, 1987.
- [21] C. Sauvaget, N. Allen, M. Hayashi, E. Spencer, J. Nagano, Validation of a food frequency questionnaire in the Hiroshima/Nagasaki life-span study, *J. Epidemiol.* 12 (2002) 394–401.
- [22] World Health Organization, International Classification of Diseases, ninth revision, WHO, Geneva, 1977.
- [23] D. Preston, J. Lubin, D. Pierce, EPICURE User's Guide, Hirosoft International Corp., Seattle, 1991.
- [24] E.A. Kordysh, I. Emerit, J.R. Goldsmith, L. Merkin, M. Quastel, A. Bolotin, M. Friger, Dietary and clastogenic factors in children who immigrated to Israel from regions contaminated by the Chernobyl accident, *Arch. Environ. Health* 56 (2001) 320–326.
- [25] S. Delanian, S. Balla-Mekias, J.L. Lefaix, Striking regression of chronic radiotherapy damage in a clinical trial of combined pentoxifylline and tocopherol, *J. Clin. Oncol.* 17 (1999) 3283–3290.
- [26] R.U. Peter, P. Gottlob, Management of cutaneous radiation injuries: diagnostic and therapeutic principles of the cutaneous radiation syndrome, *Mil. Med.* 167 (2002) 110–112.
- [27] H. Letur-Konirsch, F. Guis, S. Delanian, Uterine restoration by radiation sequelae regression with combined pentoxifylline–tocopherol: a phase II study, *Fertil. Steril.* 77 (2002) 1219–1226.
- [28] M. Kennedy, K. Bruninga, E.A. Mutlu, J. Losurdo, S. Choudhary, A. Keshavarzian, Successful and sustained treatment of chronic radiation proctitis with antioxidant Vitamins E and C, *Am. J. Gastroenterol.* 96 (2001) 1080–1084.
- [29] A. Ben-Amotz, S. Yatziv, M. Sela, S. Greenberg, B. Rachmilevich, M. Shwarzman, Z. Weshler, Effect of natural beta-carotene supplementation in children exposed to radiation from the Chernobyl accident, *Radiat. Environ. Biophys.* 37 (1998) 187–193.
- [30] I. Emerit, N. Oganessian, T. Sarkisian, R. Arutyunyan, A. Pogosian, K. Asrian, A. Levy, L. Cernjavski, Clastogenic factors in the plasma of Chernobyl accident recovery workers: anticlastogenic effect of Ginkgo biloba extract, *Radiat. Res.* 144 (1995) 198–205.
- [31] F. Gemignani, M. Ballardini, F. Maggiani, A.M. Rossi, A. Antonelli, R. Barale, Chromosome aberrations in lymphocytes and clastogenic factors in plasma detected in Belarus children 10 years after Chernobyl accident, *Mutat. Res.* 446 (1999) 245–253.

- [32] I. Emerit, M. Quastel, J. Goldsmith, L. Merkin, A. Levy, L. Cernjavski, A. Alaoui-Youssefi, A. Pogossian, E. Riklis, Clastogenic factors in the plasma of children exposed at Chernobyl, *Mutat. Res.* 373 (1997) 47–54.
- [33] C.A. Whitehouse, E.J. Tawn, No evidence for chromosomal instability in radiation workers with in vivo exposure to plutonium, *Radiat. Res.* 156 (2001) 467–475.
- [34] E.J. Tawn, C.A. Whitehouse, F.A. Martin, Sequential chromosome aberration analysis following radiotherapy—no evidence for enhanced genomic instability, *Mutat. Res.* 465 (2000) 45–51.
- [35] A.A. Awa, T. Honda, T. Sofuni, S. Neriishi, M.C. Yoshida, T. Matsui, Chromosome-aberration frequency in cultured blood-cells in relation to radiation dose of A-bomb survivors, *Lancet* 2 (1971) 903–905.
- [36] National Nutrition Survey Report, Ministry of Health and Welfare, Tokyo, 1980.
- [37] A. Okayama, H. Ueshima, M.G. Marmot, M. Nakamura, Y. Kita, M. Yamakawa, Changes in total serum cholesterol and other risk factors for cardiovascular disease in Japan 1980–1989, *Int. J. Epidemiol.* 22 (1993) 1038–1047.
- [38] A. Giacosa, R. Filiberti, M.J. Hill, J. Faivre, Vitamins and cancer chemoprevention, *Eur. J. Cancer Prev.* 6 (Suppl. 1) (1997) S47–54.
- [39] R.M. Tamimi, P. Lagiou, H.O. Adami, D. Trichopoulos, Prospects for chemoprevention of cancer, *J. Intern. Med.* 251 (2002) 286–300.
- [40] K. Arimoto, Tables of Food Composition, Daiichi Editor, Tokyo, 1960.

地域がん登録事業と今後の課題

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●はじめに

平成 15 年の人口動態統計によると、がん(悪性新生物)は死因の第 1 位であり、全死因の 30.5% を占めている。がん罹患数については人口動態統計ではなく、現在、34 道府県市で実施されている地域がん登録によって把握されている。そのうち精度の高い地域のデータをもとに、「地域がん登録精度向上と活用に関する研究」班(主任研究者:津熊秀明)によって全国罹患率が推計され、毎年報告されている。

本稿では、地域がん登録事業について、その整備の取り組み、個人情報保護とのかかわりについて紹介し、最後にその課題について述べる。

①地域がん登録事業の整備の取り組み

平成 16 年度から開始された第 3 次対がん総合戦略研究事業「がん予防対策のためのがん罹患・死亡動向の実態把握の研究」班(主任研究者:祖父江友孝)では、以下に示す 8 つの「目標と基準」を設定して地域がん登録を整備することを目指している(<http://ncrp.ncc.go.jp> 参照)。すなわち、地域がん登録が今後 10 年で達成すべき条件を「目標」、ある時点で一定水準を満たしていると判断する条件を「基準」と称し、「基準」を定期的に確認して底上げすることによって、「目標」の達成をより確実にすることとしている。

(1) がん登録事業実施に関する公的承認を得ていること

(2) がん登録に必要な項目に関して、収集・管理・提供が可能なこと

(3) 登録の完全性に関する条件を満たして

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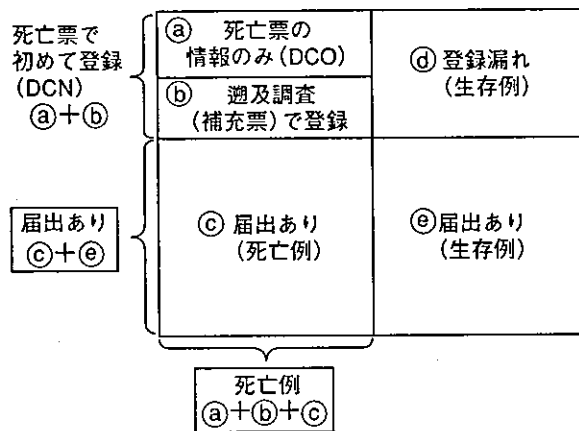


図 1 地域がん登録事業の精度に関する模式図

地域がん登録では、まず届出(地域により出張採録方式も用いられる)によってがん患者を把握し(c+e)、それを死亡票によって捕っている(a+b+c)。実際には届出漏れの生存例dが存在するが、これを除いた合計(a+b+c+e)を罹患数とする。DCO 割合とは、死亡票で初めて登録された患者(DCN; death certificate notification, 図のa+b)のうち、死亡診断書を発行した医療機関への確認調査(遡及調査)によって登録された患者数cを除いた部分、すなわち死亡票の情報だけの患者数aを罹患数(a+b+c+e)で割ったものである。

いること(登録漏れなどの量的な精度)

(4) 登録の即時性に関する条件を満たしていること(診断から集計までの迅速さ)

(5) 登録の品質に関する条件を満たしていること(登録項目における不詳割合の低さなどの質的な精度)

(6) 予後追跡調査を行い、追跡率が条件を満たしていること

(7) 報告書作成を行っていること

(8) 登録資料の研究的利用の手続きが整備されていること(資料の研究的利用)

冒頭で述べた「精度の高い」地域がん登録事業とは、「目標と基準(3)」にある登録の完全性をもとに選ばれている。完全性の指標として DCO

(death certificate only)割合が用いられ、DCO割合が25%未満で精度が良いとされている(図1)。

②地域がん登録事業と個人情報保護のかかわり

地域がん登録事業は昭和57年に制定された老人保健法により、都道府県の行うべき事業と規定され(健医老第68号)、罹患率、受療状況、生存率等の集計、解析によって、各地域のがんの動向を把握し、さらに市町村で実施されるがん検診事業の評価を行うこととなった。その後、平成15年5月に施行された健康増進法第16条により、「(生活習慣病の発生状況の把握)国及び地方公共団体は、生活習慣とがん、循環器病その他の生活習慣病との相関関係を明らかにするため、生活習慣病の発生状況の把握に努めること」、具体的な内容は、「地域がん登録事業及び脳卒中登録事業であること」として、一定の法的根拠を与えられるに至った。

地域がん登録では、ある地域で発生したがん症例を対象としているため、さまざまな医療機関から届出された情報をもとに同一の腫瘍かどうか、その個人を同定する必要がある。そのため個人識別情報(氏名、年齢、住所など)は必須である。そこで、プライバシー保護の観点から検討を重ね、平成8年に厚生省がん研究助成金「地域がん登録の精度向上と活用に関する研究」班(主任研究者:花井 彩)では、ガイドライン「地域がん登録における情報保護」を策定し、機密保持に十分に努めてきた。

平成14年6月には「疫学研究に関する倫理指針」(文部科学省・厚生労働省)により、個人情報を扱う場合にはインフォームド・コンセントが必要であることが示されたが、別添の「『疫学研究に関する倫理指針』とがん登録事業の取扱いについて」で「がん登録事業については、本指針は適用されない」と明記され、保健事業として従来どおり事業を実施することが可能となった。

しかし個人情報保護法、具体的には「個人情報の保護に関する法律(平成15年法律第57号)」、「行政機関の保有する個人情報の保護に関する法律(平成15年法律第58号)」および「独立行政法人等の保有する個人情報の保護に関する法律(平成15年法律第59号)」が平成15年5月に制定され、基本的に個人情報を扱う場合には対象者からインフォームド・コンセントを得ることが必須となった。

がん登録事業は、がん告知をされていない者や死亡した者からインフォームド・コンセントを得ることが難しいこと、また個人に不利益を及ぼすことがないこと、そして何よりも公衆衛生の推進のために必要な事業であることから、これらの法律の適用除外となることが望まれた。そのため、平成16年1月8日付けで厚生労働省健康局長通知(健習発第0108003号)が都道府県知事、政令市長、特別区長宛てに出され、「法令に従い個人情報の保護に十分な配慮をすることが重要であるが、地域がん登録事業の取扱いについては、利用目的による制限や第三者提供の制限の適用除外の事例に該当する」となった。なお本通知においても、前述のガイドライン「地域がん登録における情報保護」が別添として示されている。

●おわりに

地域がん登録事業は世界的にもがん罹患数の集計等に重要な役割を果たしている。このがん罹患数把握をさらに充実させるためにも、地域がん登録は院内がん登録と連携した形で今後、整備が進められることが必要である。また、地域がん登録事業はまだすべての都道府県で実施されているわけではなく、がん罹患数の全国値を完全に把握できているわけではない。がん罹患数を精巧かつ迅速性をもって把握するために、個人情報保護への対策も配慮した法的整備が求められている。

Effects of Dementia on Mortality in the Radiation Effects Research Foundation Adult Health Study

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Key Words

Alzheimer's disease · Vascular dementia · Mortality · Longitudinal study · Japan

Abstract

Background: Although dementia is rarely listed on death certificates, it does contribute to mortality. The predominant immediate causes of death coincident with dementia are pneumonia and cardiovascular diseases. **Objective:** To estimate the impact of dementia on specific mortality risks. **Methods:** We applied DSM-III/R criteria for Alzheimer's disease (AD) and vascular dementia (VaD) to 2,172 subjects of the Adult Health Study of the Radiation Effects Research Foundation who were 60 or more years old when examined from 1992 to 1996. The underlying causes of death were compiled from death certificates. We performed a Poisson regression analysis to evaluate specific causes of mortality for which AD or VaD was a significant risk factor. **Results:** The relative risk of mortality was 2.2 for AD and 2.4 for VaD. Mortality from pneumonia and stroke was elevated for both types of dementia, independent of other medical conditions. AD was also associated with death from trauma. **Conclusion:** Dementia was a predictor of death due to pneumonia, stroke, and trauma among the Japanese elderly. The

prevention and early detection of those conditions are important in the medical care and treatment of dementia cases.

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Introduction

Although relatively few death certificates report dementia [1, 2], it is a cause of increased mortality according to longitudinal studies in Europe [3, 4], the US [5], and Japan [6, 7]. Pneumonia and cardiovascular diseases are the predominant immediate causes of dementia-associated deaths [8–11]. Few reports evaluate the impact of dementia on specific disease risks. The purpose of this study was to estimate the impact of dementia on specific risks of mortality in the Radiation Effects Research Foundation (RERF) Adult Health Study (AHS) population. In the AHS, prevalence cases of dementia, based on the *Diagnostic and Statistical Manual of Mental Disorders*, 3rd edition, revised (DSM III/R) were identified between 1992 and 1996 [12]. The prevalence of dementia in the cohort was 7.2% and was thus similar to that in the general population [12]. Ascertainment of vital status in the cohort is essentially completed, and death certificates were consulted to determine the causes of death.

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Table 1. Study subjects by sex, age, and DSM III/R diagnosis

Sex	DSM III/R	Age				Total
		60-69	70-79	80-89	≥ 90	
Male	Non-dementia	371	132	84	8	595
	AD	1	2	7	3	13
	VaD	5	4	4	0	13
Female	Non-dementia	636	566	233	27	1,462
	AD	1	11	38	11	61
	VaD	3	10	12	3	28
Total		1,017	725	378	52	2,172

Table 2. RR and 95% CI derived from Poisson regression analysis

Cause of death	Total		Stroke		Ischemic heart disease		Pneumonia		Trauma	
	RR	95% CI	RR	95% CI	RR	95% CI	RR	95% CI	RR	95% CI
Sex (female/male)	0.44	0.36-0.55**	0.51	0.30-0.91*	0.50	0.23-1.21	0.26	0.14-0.49**	0.80	0.21-3.81
Age (5 year increments)	1.66	1.56-1.78**	1.61	1.37-1.92**	1.91	1.49-2.48**	1.96	1.60-2.43**	1.12	0.73-1.69
Medical history										
Heart disease	1.41	1.07-1.83*	0.84	0.35-1.75	4.16	1.85-9.10**	0.92	0.34-2.07	5.47	0.04-4.09
Stroke	1.48	1.05-2.06*	3.46	1.67-6.67**	1.28	0.27-4.08	0.64	0.18-1.76	5.37	0.90-23.7*
Cancer	1.47	1.06-2.00*	0.55	0.13-1.52	1.31	0.30-3.89	2.27	0.84-5.22	-	-
Dementia										
AD	2.17	1.52-3.06**	2.73	1.12-6.06*	1.54	0.34-4.97	5.90	2.62-12.9**	9.56	1.14-61.5*
VaD	2.38	1.41-3.87**	2.50	0.85-6.49	1.17	0.06-7.05	12.30	3.47-36.7**	2.49	0.12-21.9

* p < 0.05, ** p < 0.01.

Methods

The study subjects consisted of 2,172 subjects of the AHS in Hiroshima, 60 or more years old, who underwent screening for cognitive impairment between September 1992 and September 1996 [12]. The study was approved by the Human Investigation Committee at RERF, and all subjects gave informed consent before being examined. Based on DSM III/R criteria applied to cognitive function tests, neurological examinations, and informant questionnaires, 74 cases of Alzheimer's disease (AD) and 41 of vascular dementia (VaD) were identified, while 2,057 subjects did not have dementia. Table 1 shows the study subjects by sex, age at baseline examination, and dementia diagnosis. The cohort was followed up until December 31, 1999, or until death if it occurred earlier. Deaths were confirmed by the Japanese family registration system. The causes of death were identified through death certificates and classified on the basis of the International Classification of Diseases (ICD). After adjusting for sex, age at baseline examination, and disease history (heart disease, stroke, and cancer), we performed Poisson regression analysis to determine whether AD or VaD was a significant risk factor for mortality from all causes, ischemic heart disease, stroke, pneumonia, and trauma. Disease histories were obtained from AHS health examinations that included history-taking, physical examination, and laboratory tests.

Results

During the follow-up period, 284 (13.8%) of the non-dementia cases, 43 (58.1%) of the AD cases, and 21 (51.2%) of the VaD cases died. The average follow-up periods for non-dementia, AD, and VaD were 5.2, 3.8, and 4.0 years, respectively. Among non-dementia cases, the predominant causes of death were cancer (33%), stroke (14%), pneumonia (9%), and ischemic heart disease (8%). In the combined AD and VaD cases, the predominant causes of death were pneumonia and stroke; 3 death certificates named dementia as the underlying cause of death. Among AD cases, the predominant causes of death were pneumonia (28%), stroke (19%), ischemic heart disease (7%), dementia (7%), trauma (5%), senility (5%), and others. Among VaD cases, the causes of death were stroke (29%), pneumonia (24%), senility (14%), ischemic heart disease (5%), trauma (5%), and others. Table 2 shows the results of Poisson regression analysis. Dementia increased the risk of mortality independently

of age at baseline, sex, and comorbidity. Relative risk (RR) of total mortality was 2.2 for AD and 2.4 for VaD. For both AD and VaD, mortality from pneumonia and stroke increased significantly. RR of mortality from stroke was 2.7 for AD and 2.5 for VaD, and RR of mortality from pneumonia was 5.9 for AD and 12.3 for VaD. AD also increased the risk of mortality from trauma significantly (RR = 9.6). There was no statistically significant difference of mortality from ischemic heart disease between dementia and non-dementia cases.

Discussion

This is one of the few population-based studies in which mortality associated with dementia has been investigated after adjustment for other medical conditions. Although dementia is rarely listed on death certificates [1, 2], dementia increases the risk of mortality significantly [3–5]. The RR of 2.2 for AD and 2.4 for VaD that we report here are close to those reported for community-based studies in the US and Europe, where the RR of mortality is 1.9–3.6 for dementia and 1.4–3.3 for AD [3–5]. Although dementia presents a significant risk for mortality, little is known about its impact on specific mortality risks. Burns et al. [9] and Keene et al. [11], using death certificate diagnoses, reported that the main immediate causes of death among subjects with dementia recorded at autopsy are pneumonia and cardiovascular disease, which is in agreement with our study. Among the AHS cohort, the RR of death from pneumonia was significantly higher for subjects with AD and VaD than for those without dementia, and it was higher than that of cancer. This

result is compatible with the report by Morrison and Siu [10], who reported that patients with advanced dementia and pneumonia had a poor prognosis. In a previous study of the AHS population, the prevalence of VaD, but not AD, increased with a history of stroke [12], while in the present study, mortality from stroke increased not only in VaD but also in AD. Since many VaD patients had a history of stroke, RR of death from stroke among VaD patients may be close to the product of the risks.

Although stroke events among 7 AD patients occurred after the onset of dementia, the increased mortality from stroke in AD suggests that the etiology of AD may include a vascular component. Most demented subjects near the end of life have difficulty with the basic activities of daily living [1], and the increased mortality from trauma associated with AD might be caused by disabilities associated with dementia. Since RR of death from trauma among VaD patients is thought to be close to the product of the risks of stroke history and VaD, the increased mortality from trauma may be associated with VaD as well.

Increased mortality from pneumonia, stroke, and trauma was observed in demented subjects independent of other relevant medical conditions. Thus, the prevention and early detection of those events are necessary in the medical care and treatment of dementia cases.

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References

- 1 Lanska D: Dementia mortality in the United States. Results of the 1986 National Mortality Followback Survey. *Neurology* 1998;50:362–367.
- 2 Losonczy KG, White LR, Brock DB: Prevalence and correlates of dementia: Survey of the last days of life. *Public Health Rep* 1998;113:273–280.
- 3 Agüero-Torres H, Fratiglioni L, Guo Z, Vitanen M, Winblad B: Mortality from dementia in advanced age: A 5-year follow-up study of incident dementia cases. *J Clin Epidemiol* 1999;58:737–743.
- 4 Baldereschi M, Di Carlo A, Maggi S, Grigoletto F, Scarlato G, Amaducci L, Inzitari D: Dementia is a major predictor of death among the Italian elderly. *Neurology* 1999;52:709–713.
- 5 Evans DA, Smith LA, Scherr PA, Albert MS, Funkenstein HH, Hebert LE: Risk of death from Alzheimer's disease in a community population of older persons. *Am J Epidemiol* 1991;134:403–412.
- 6 Washio M, Nohtomi A, Ikeda M, Koga H, Hamada T, Okayama M, Yoshimura T, Fujishima M: Dementia and other risk factors for mortality in the institutionalized Japanese elderly. *J Epidemiol* 1995;5:117–123.
- 7 Hasegawa K, Homma A, Yook M: A gerontopsychiatric 5-year follow-up study on age-related dementia in those residing in the community. *Jpn J Geriatr* 1980;17:630–638.
- 8 Boersma F, Van Den Brink W, Deeg DJ, Eefsting JA, Van Tilburg W: Survival in a population-based cohort of dementia patients: Predictors and causes of mortality. *Int J Geriatr Psychiatry* 1999;14:748–753.
- 9 Burns A, Jacoby R, Luthert P, Levy R: Cause of death in Alzheimer's disease. *Age Aging* 1990;19:341–344.
- 10 Morrison RS, Siu AL: Survival in end-stage dementia following acute illness. *JAMA* 2000;284:47–52.
- 11 Keene J, Hope T, Fairburn CG, Jacoby R: Death and dementia. *Int J Geriatr Psychiatry* 2001;16:969–974.
- 12 Yamada M, Sasaki H, Mimori Y, Kasagi F, Sudoh S, Ikeda J, Hosada Y, Nakamura S, Kodama K: Prevalence and risks of dementia in the Japanese population: RERF's Adult Health Study Hiroshima subjects. *J Am Geriatr Soc* 1999;47:189–195.

LTA 252G allele containing haplotype block is associated with high serum C-reactive protein levels

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Abstract

C-reactive protein (CRP), an inflammatory biomarker, is a predictor of future risk for cardiovascular disease. Hypothetically, the levels of inflammatory response to microbial and lifestyle-related factors are influenced by genetic factors. LT- α is a proinflammatory cytokine that plays an important role in the pathogenesis of atherosclerosis in mice. We examined the association between gene polymorphism of the LT- α coding gene, *LTA* A252G, and CRP based on a case–control study. The top 149 and bottom 151 subjects in terms of CRP levels were selected for genotyping from among 1000 A-bomb survivors free from acute infection, chronic liver diseases, uremia, autoimmune diseases, and cancers. The genotype of *LTA* was determined by fluorescence resonance energy transfer–polymerase chain reaction (FRET–PCR) and subsequent melting curve analysis. The values of traditional risk factors such as body mass index (BMI), white blood cell (WBC) count, hemoglobin (Hb) concentration, and glycated Hb (HbA1c) differed significantly between the low and high CRP groups. After adjusting for the effect of sex, age, BMI, WBC, Hb, and HbA1c, the *LTA* 252G allele was found to be associated with high CRP levels (odds ratio = 1.93, $P = 0.007$) by multiple logistic regression analysis. Thus, CRP levels are influenced not only by environmental factors but also by the polymorphism of *LTA* or other genes in the same haplotype block.

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Keywords: Inflammation; Interleukins; Risk factors; Atherosclerosis; Genetics

1. Introduction

Atherosclerosis is thought to be an inflammatory disease [1]. A number of studies have examined various circulation biomarkers of inflammation as potential predictors of future risk for cardiovascular disease. Among them, C-reactive protein (CRP) is one of the most consistent biomarkers in a variety of clinical settings of risk for cardiovascular disease [2–4]. In addition to microbial infection in arterial walls [5,6], traditional cardiovascular risk factors such as white blood cell (WBC) count, body mass index (BMI), age, and smoking were associated with high CRP in healthy individuals [7], indicating that these lifestyle-related factors also trigger low-grade inflammation. The biological significance of high CRP must be considered cautiously; however, it may

reflect high levels of environmental stimuli that trigger inflammation, or possibly high responsiveness to such stimuli, possibilities that are not mutually exclusive.

LT- α (TNF- β) and TNF- α are proinflammatory cytokines coded for by the *LTA* and *TNFA* genes, respectively. Both cytokines induce apoptosis in cells upon binding to TNF receptor type 1, whereas they induce inflammatory responses by activating NF- κ B nuclear protein upon binding to TNF receptor type 2 [8]. Thus, the cytokines have the potential to boost inflammatory response. Moreover, both cytokines are expressed in atherosclerotic lesions [9,10]. A recent report indicates that atherosclerosis in atherogenic diet-fed mice was attenuated by the disruption of the *LTA* gene but not the *TNFA* gene [10]. This report prompted us to investigate the hypothesis that the atherosclerosis-related gene *LTA* in mice may influence serum CRP levels in healthy individuals. Here we report that the *LTA* 252G allele is associated with high CRP levels in healthy human subjects.

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2. Materials and methods

Subjects in this study were selected from among 1000 members of the Adult Health Study (AHS) longitudinal cohort established in 1958 who received medical examinations in Hiroshima from October 1999 to January 2001 and did not suffer from acute infection, chronic liver diseases, uremia, autoimmune diseases, or cancers. The AHS cohort is composed of four different radiation exposure categories, from zero- to high-dose groups [11]. Serum CRP levels were measured with a highly sensitive CRP-Latex kit using anti-CRP monoclonal antibodies (Nissui Pharmaceutical Co. Ltd., Tokyo, Japan). The top 149 and bottom 151 subjects in terms of serum CRP levels were selected for the present study. There was no difference between the two groups ($P = 0.68$) in the prevalence of cardiovascular disease (ICD code—ninth: 401–405, 410–414; tenth: I10–I15, I20–I25). Preliminary analyses demonstrated that radiation dose distribution did not significantly differ among low and high CRP groups ($P = 0.2644$ by Student's *t*-test) or three *LTA* genotype groups ($P = 0.0814$ by Student's *t*-test) in the present subjects. Thus, we did not include dose as a variable in the present analysis.

Written informed consent regarding genome research was obtained from the participants according to the Guidelines for Genome/Genetic Research issued by the Japanese government. RERF's Ethical Committee for Genome/Genetic Research approved this research protocol in our institute (pilot study no. B25).

Genomic DNA was extracted from whole blood using the QIAamp[®] DNA Blood Mini Kit (QIAGEN). The genotype of *LTA* was determined by fluorescence resonance energy transfer–polymerase chain reaction (FRET–PCR) technique with subsequent melting curve analysis using a real-time PCR instrument (Light Cycler[®], Roche Diagnostics) [12]. PCR primers and fluorogenic probes used were: forward primer (5'-AGAGAAGGGGACAAGATGCAGT-3'), reverse primer (5'-GGCCTTGGTGGGTTTGGTT-3'), sensor probe (5'-CAGAGAGGAACCATGGCAGA-3'-FITC) and anchor probe (Red640-5'-CAGAGAATGTGTGACAGAGACAAT-3'). The thermocycling procedure consisted of 45 cycles of denaturation at 95 °C for 15 s, annealing at 65 °C for 15 s, and extension at 72 °C for 15 s using 3 mM MgCl₂. After amplification, a melting curve analysis was performed by changing the temperature from 95 °C for 1 s to 40 °C for 15 s, and then gradually raising the temperature to 70 °C over a period of 7 min. Accuracy of genotyping using the FRET–PCR method was validated in 21 cases by comparing the results with sequence data obtained by a PCR-based direct sequencing method.

2.1. Statistical analyses

Genotype frequencies of the subjects with high CRP levels were compared with those of the subjects with low CRP levels, after adjustment for potential confounders that included

sex, age at sampling, BMI, WBC, Hb, HbA1c, total cholesterol, and smoking status. We computed the unadjusted and adjusted odds ratios (ORs) and their 95% confidence intervals (CIs) using multiple logistic regression models. The distribution of the *LTA* A252G genotype in the low CRP group corresponded well with the Hardy-Weinberg equilibrium ($P = 0.31$ by χ^2 statistics). All calculations were performed with STATA8 (Stata Corporation, College Station, TX).

3. Results

Basic characteristics of the high CRP and low CRP groups are shown in Table 1. Significant differences between the high and low CRP groups were observed in the levels of traditional risk factors for cardiovascular disease such as BMI, WBC, Hb, and HbA1c, but not in the distribution of sex, age, smoking, and total cholesterol. As mentioned in Section 2, radiation dose at the time of the A-bombings was not associated with either CRP or certain genotypes, and therefore we did not adjust for the dose in the following analyses.

Before adjustment for traditional cardiovascular risk factors, *LTA* 252G carriage was associated with high CRP (OR = 1.84, 95% CI = 1.13–3.02 for GA; OR = 2.08, 95% CI = 1.01–4.25 for GG genotype) (Table 2). After adjustment for traditional risk factors associated with high CRP (Table 2), the association remained between the *LTA* 252G allele and high CRP (OR = 1.93, 95% CI = 1.14–3.29), indicating that this positive association might not be affected by the traditional cardiovascular risk factors BMI, WBC, Hb, and HbA1c.

Table 1
Basic characteristics of subjects

	High CRP	Low CRP	<i>P</i> -value ^a
Sex			
Male (%)	62 (41.6%)	54 (35.8%)	0.30
Female (%)	87 (58.4%)	97 (64.2%)	
Cardiovascular disease	46 ^b (30.9%)	50 ^b (33.1%)	0.68
Smoking status			
No (%)	106 (71.1%)	109 (72.2%)	0.84
Yes (%)	43 (28.9%)	42 (27.8%)	
	Mean (S.D.)		
CRP (mg/dl)	0.254 (0.33)	0.009 (0.01)	<0.0001
Age (year)	59.8 (4.8)	59.2 (5.1)	0.26
BMI (kg/m ²)	24.4 (3.4)	22.0 (2.7)	<0.0001
WBC ($\times 10^2/\mu$ l)	62.5 (18.0)	53.6 (13.3)	<0.0001
Hemoglobin (g/dl)	13.8 (1.4)	13.4 (1.2)	0.008
HbA1c (%)	5.7 (1.1)	5.2 (0.7)	0.0001
Total cholesterol (mg/dl)	218.7 (35.0)	220.6 (37.1)	0.66

S.D.: standard deviation.

^a *P*-values from χ^2 -test for group variables and *P*-values from unpaired *t*-test with unequal variance for continuous variables.

^b Eight subjects each in the high and low CRP groups had coronary heart disease, and the others had hypertension.

Table 2
LTA 252G allele is dominantly associated with high CRP

	Low CRP no. (%)	High CRP no. (%)	Unadjusted			Adjusted		
			Odds ratio	95% CI	χ^2 (P-value)	Odds ratio ^a	95% CI	χ^2 (P-value)
Genotype								
AA	75 (49.7)	51 (34.2)	1.00		7.48	1.00		6.02
AG	59 (39.1)	74 (49.7)	1.84	(1.13–3.02)	(0.024)	1.94	(1.10–3.41)	(0.049)
GG	17 (11.3)	24 (16.1)	2.08	(1.01–4.25)		1.93	(0.85–4.37)	
Allele carriage								
AA	75 (49.7)	51 (34.2)	1.00		7.34	1.00		6.02
AG + GG	76 (50.3)	98 (65.8)	1.90	(1.19–3.02)	(0.007)	1.93	(1.14–3.29)	(0.014)

^a Baseline odds were adjusted for sex, age, BMI, WBC, Hb, and HbA1c.

4. Discussion

Inflammation's key role in the pathogenesis of atherosclerosis is well established. Several possible mechanisms have been theorized: microbial infection in atherosclerotic plaque [5,6], inflammatory reaction caused by oxidative stress [13,14], and production of the inflammatory cytokine IL-6 by adipose tissue [15]. Thus, both microbes and lifestyle-related factors seem to trigger CRP production. Increasing evidence in different clinical situations has demonstrated that CRP is among the most consistent biomarkers for future risk of cardiovascular disease [2–4]. However, little is known about the effect of genetic factors on CRP in response to various stimuli.

Many polymorphic genes regulate inflammation and immune response. In this context, the TNF family of genes is of interest for three reasons. First, TNF- α and LT- α proteins are proinflammatory cytokines that are expressed in atherosclerotic lesions [9,10]. Second, the polymorphisms of *TNFA* and *LTA* are associated with levels of cytokine production and immune response [16]. Third, disruption of the *LTA* gene but not the *TNFA* gene attenuated atherosclerosis in atherogenic diet-fed mice [10]. Thus, *LTA* seems to play an important role in atherosclerosis, at least in mice. LT- α is a proinflammatory cytokine that induces inflammatory cytokines as well as acute-phase proteins such as CRP by inducing the nuclear factors NF κ B and NF-IL6 [17,18]. In the present study, we clearly demonstrated a significant association between the *LTA* 252G allele and high CRP levels (Table 2). The result is concordant with Messer's report that the *LTA* 252G homozygote was associated with high LT- α production by phytohaemagglutinin-activated mononuclear cells [16]. The *LTA* 252G allele elevated CRP independent of the traditional cardiovascular risk factors BMI, WBC, Hb, and HbA1c (Table 2). Thus, these traditional risk factors might elevate CRP through molecular mechanisms other than LT- α induction.

Ozaki et al. recently reported an association between the *LTA* 252G allele and susceptibility to myocardial infarction [19]. The *LTA* 252G allele is completely linked to polymorphisms of two other genes in the Japanese population: the –63A allele of NF κ B inhibitor-like 1 gene (*NFKBIL1*) and

the –23C allele of *BAT119*. The –63A allele of *NFKBIL1* was reported to be associated with reduced transcriptional activity of the gene [19]. The physiological significance of *NFKBIL1* polymorphism in vivo is not clear, it might potentially affect inflammatory response through altering the translocation of nuclear protein NF κ B from cytoplasm to nucleus. *BAT1* is a member of the DEAD-box family of RNA-dependent ATPases. Inactivation of *BAT1* resulted in the higher production of proinflammatory cytokines in cultured cells [20], indicating a negative regulatory role for *BAT1*. Since the –23C allele of *BAT1* was suspected to reduce gene expression [21], it might affect the levels of proinflammatory cytokines in vivo. Although *NFKBIL1* –63A, *BAT1* –23C and *LTA* 252G alleles in the same haplotype block may potentially affect the levels of CRP, it is premature to conclude which gene polymorphism is dominant in terms of increased CRP levels. Future studies will elucidate the specific gene polymorphism that affects CRP levels.

In the present study, CRP was not associated with the prevalence of cardiovascular disease, although CRP is a predictor for future risk of coronary heart disease. In Japan, the prevalence of myocardial infarction was low compared with that in the US [22]. Most subjects with cardiovascular disease in the present study were those with hypertension, and only eight subjects each in the low and high CRP groups had coronary heart disease. Those numbers were too small to demonstrate an association between CRP and coronary heart disease.

The subjects in the present study were selected from the cohort of atomic bomb survivors. There is a concern that the cohort might be biased in terms of survivability in an atomic bombing disaster. We have to be cautious about whether gene polymorphisms potentially associated with survivability are overly represented in A-bomb survivors. Our cohort consists of subjects with different radiation exposure categories, from zero to high dose, which enables us to assess the association between dose and genotype. As mentioned in Section 2, there was no significant association between *LTA* genotype and radiation dose. Thus, we believe that the present results are not biased in the selection of samples.

This study is the first to demonstrate that the levels of CRP in healthy individuals are influenced not only by

environmental factors but also by a genetic factor, i.e. the polymorphism of *LTA* or closely linked genes.

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References

- [1] Ross R. Atherosclerosis—an inflammatory disease. *N Engl J Med* 1999;340:115–26.
- [2] Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. *N Engl J Med* 1997;336:973–9.
- [3] Kuller LH, Tracy RP, Shaten J, Meilahn EN. Relation of C-reactive protein and coronary heart disease in the MRFIT nested case-control study. Multiple risk factor intervention trial. *Am J Epidemiol* 1996;144:537–47.
- [4] Haverkate F, Thompson SG, Pyke SD, Gallimore JR, Pepys MB. European Concerted Action on Thrombosis and Disabilities Angina Pectoris Study Group. Production of C-reactive protein and risk of coronary events in stable and unstable angina. *Lancet* 1997;349:462–6.
- [5] Kalayoglu MV, Libby P, Byrne GI. *Chlamydia pneumoniae* as an emerging risk factor in cardiovascular disease. *J Am Med Assoc* 2002;288:2724–31.
- [6] Witherell HL, Smith KL, Friedman GD, et al. C-reactive protein, *Helicobacter pylori*, *Chlamydia pneumoniae*, cytomegalovirus and risk for myocardial infarction. *Ann Epidemiol* 2003;13:170–7.
- [7] Saito M, Ishimitsu T, Minami J, Ono H, Ohrui M, Matsuoka H. Relations of plasma high-sensitivity C-reactive protein to traditional cardiovascular risk factors. *Atherosclerosis* 2003;167:73–9.
- [8] Lucas R, Lou J, Morel DR, Ricou B, Suter PM, Grau GE. TNF receptors in the microvascular pathology of acute respiratory distress syndrome and cerebral malaria. *J Leukocyte Biol* 1997;61:551–8.
- [9] Kaartinen M, Penttila A, Kovanen PT. Mast cells in rupture-prone areas of human coronary atheromas produce and store TNF- α . *Circulation* 1996;94:2787–92.
- [10] Schreyer SA, Vick CM, LeBoeuf RC. Loss of lymphotoxin- α but not tumor necrosis factor- α reduces atherosclerosis in mice. *J Biol Chem* 2002;277:12364–8.
- [11] Wong FL, Yamada M, Sasaki H, et al. Noncancer disease incidence in the atomic bomb survivors, 1958–1986. *Radiat Res* 1993;135:418–30.
- [12] Grosch S, Niederberger E, Lotsch J, Skarke C, Geisslinger G. A rapid screening method for a single nucleotide polymorphism (SNP) in the human MOR gene. *Br J Clin Pharmacol* 2001;52:711–4.
- [13] Davi G, Guagnano MT, Ciabattini G, et al. Platelet activation in obese women: role of inflammation and oxidant stress. *J Am Med Assoc* 2002;288:2008–14.
- [14] Riserus U, Basu S, Jovinge S, Fredrikson GN, Arnlov J, Vessby B. Supplementation with conjugated linoleic acid causes isomer-dependent oxidative stress and elevated C-reactive protein: a potential link to fatty acid-induced insulin resistance. *Circulation* 2002;106:1925–9.
- [15] Yudkin JS, Kumari M, Humphries SE, Mohamed-Ali V. Inflammation, obesity, stress and coronary heart disease: is interleukin-6 the link? *Atherosclerosis* 2000;148:209–14.
- [16] Messer G, Spengler U, Jung MC, Honold G, Blomer K, Pape GR, et al. Polymorphic structure of the tumor necrosis factor (TNF) locus: an NcoI polymorphism in the first intron of the human TNF- β gene correlates with a variant amino acid in position 26 and a reduced level of TNF- β production. *J Exp Med* 1991;173:209–19.
- [17] Akira S, Kishimoto T. IL-6 and NF-IL6 in acute-phase response and viral infection. *Immunol Rev* 1992;127:25–50.
- [18] Li SP, Goldman ND. Regulation of human C-reactive protein gene expression by two synergistic IL-6 responsive elements. *Biochemistry* 1996;35:9060–8.
- [19] Ozaki K, Ohnishi Y, Iida A, Sekine A, Yamada R, Tsunoda T, et al. Functional SNPs in the lymphotoxin- α gene that are associated with susceptibility to myocardial infarction. *Nat Genet* 2002;32:650–4.
- [20] Allcock RJ, Williams JH, Price P. The central MHC gene, *BAT1*, may encode a protein that down-regulates cytokine production. *Genes Cells* 2001;6:487–94.
- [21] Wong AM, Allcock RJ, Cheong KY, Christiansen FT, Price P. Alleles of the proximal promoter of *BAT1*, a putative anti-inflammatory gene adjacent to the TNF cluster, reduce transcription on a disease-associated MHC haplotype. *Genes Cells* 2003;8:403–12.
- [22] Worth RM, Kato H, Rhoads GG, Kagan K, Syme SL. Epidemiologic studies of coronary heart disease and stroke in Japanese men living in Japan, Hawaii and California: mortality. *Am J Epidemiol* 1975;102:481–90.

An Association between Oxidative Stress and Radiation-Induced Lymphomagenesis

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It is generally thought that reactive oxygen species (ROS) play an important role in carcinogenesis. However, direct evidence supporting this idea is still lacking. In the present study, we measured ROS in thymocytes at the thymic prelymphoma stage in C57BL/6 mice. Mice ($n = 20$) were irradiated at 1.6 Gy/week for 4 consecutive weeks and the levels of ROS were measured 8 to 11 weeks later by dehydrorhodamine 123, which accumulated in mitochondria and became fluorescent dye upon oxidation. Unirradiated littermates ($n = 17$) served as controls. Thymic prelymphoma cells were diagnosed by the aberrant CD4/CD8 staining profile and monoclonal or oligoclonal T-cell receptor gene rearrangement. A significant fraction of mice (11/13) bearing thymic prelymphoma cells exhibited elevated levels of ROS in thymocytes ($P < 0.001$). The result is consistent with the hypothesis that ROS may play an important role in radiation carcinogenesis. © 2004 by Radiation Research Society

INTRODUCTION

There is increasing evidence that most cancers contain multiple mutations, and the accumulation of dysfunction in several key molecules seems essential for carcinogenesis (1). In normal cells, the mutation rate is low, of the order of 10^{-10} mutations per nucleotide per cell per generation, which is insufficient to account for the large numbers of mutations observed in cancer cells (2). It is believed that only cells with mutator phenotypes or genomic instability will accumulate genetic alterations required for carcinogenesis. Radiation is a well-known mutagen that induces many kinds of neoplasms (3). Radiation can acutely induce DNA double-strand breaks, single-strand breaks, and crosslinking between DNA and protein in a dose-dependent manner (4).

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The impact of these alterations on cells may either initiate the process of multistage carcinogenesis in stem cells or accelerate the process already initiated in cells by other mutagens. Furthermore, radiation is believed to have a long-lasting effect on the progeny of irradiated cells by inducing genomic instability, and it may shorten the period required for the progression of each step in multistage carcinogenesis (5–7). However, the molecular mechanisms of induction of genomic instability by radiation have not been fully elucidated.

Reactive oxygen species (ROS) are generated constantly in cells and injure DNA in the nucleus as well as deoxy-nucleotide triphosphate in its cellular pool. Y-family polymerases erroneously incorporate an oxidized deoxynucleotide into DNA or repair an oxidized DNA erroneously (8, 9). Thus an increase in the production of ROS may cause genomic instability and facilitate the process of multistage carcinogenesis. In support of this idea, mice with a mutation in the cytochrome b subunit exhibited an increase in ROS production and a high mutation rate.² Recently, it became evident that radiation elevates ROS in the progeny of irradiated cells *in vitro* (10–12), which forced us to test the hypothesis that one of the mechanisms of radiation-induced genomic instability might be the prolonged elevation of ROS *in vivo*. To test this, we took advantage of a radiation-induced thymic lymphoma model in which more than 90% of irradiated B10 mice generated thymic lymphoma (13), and thymic prelymphoma cells could be detected by the presence of oligoclonal T-cell receptor (TCR) gene rearrangement in the thymus. ROS were measured directly in mitochondria by the oxidation-sensitive fluorescent probe dihydrorhodamine 123 (DHR) (10). Our results clearly demonstrated significant associations of elevated oxidative stress with aberrant CD4/CD8 staining profile and mono- or oligoclonal TCR gene rearrangement in thymocytes at the thymic prelymphoma stage in irradiated mice. This information supported the hypothesis that an increase in generation of ROS might be one of the mech-

² N. Ishii, Tokai University School of Medicine, Kanagawa, Japan, personal communication.

anisms of radiation-induced genomic instability that could lead to lymphomagenesis.

MATERIALS AND METHODS

Mice

C57BL/6 mice were bred and kept in our conventional animal facility in the National Institute of Radiological Sciences, Chiba. Four-week-old female mice were X-irradiated with 1.6 Gy four times for 4 consecutive weeks using a Pantak X irradiator (Pantak Ltd., East Haven, CT) at 200 kVp, 20 mA with 0.5 mm copper and aluminum filters. Unirradiated female littermates served as controls. Several weeks after irradiation, mice were transferred to the Radiation Effects Research Foundation, Hiroshima, and kept in a conventional animal facility. Three to five irradiated mice and unirradiated littermates were killed humanely 8 to 11 weeks after the last irradiation, as indicated. The chairman of the institutional animal use committee reviewed and assured that all experiments had been done following the International Guiding Principles for Biomedical Research Involving Animals issued by WHO in 1985.

Flow Cytometry Analyses

Thymocytes (1×10^6) were suspended in 1 ml of RPMI-1640 medium supplemented with 2% fetal bovine serum. Cells were stored in the dark and incubated with DHR (Molecular Probes, Eugene, OR) at a final concentration of $1 \mu\text{M}$ at 37°C for 80 min. DHR is a pro-fluorescent dye that accumulates in mitochondria and becomes fluorescent rhodamine-123 upon oxidation by ROS. After washing once with medium, cells were analyzed by flow cytometry (FACScan Becton Dickinson, Mountain View, CA). The mean fluorescence intensity was calculated using CellQuest software (Becton Dickinson). Thymocytes were stained with PE-conjugated anti-CD4 and FITC-conjugated anti-CD8 and analyzed with the FACScan. In the case of three-color staining, cells incubated with DHR were further reacted with CyChrome-conjugated anti-CD4 and PE-conjugated anti-CD8. All fluorochrome-labeled reagents were purchased from PharMingen (San Diego, CA).

TCR Gene Rearrangement

Genomic DNA was extracted from thymocytes using Qiagen Genomic-tip 100 (Qiagen, Tokyo). Specific primers and PCR conditions for the amplification of the genomic DNA segment containing TCR D β and J β genes were reported elsewhere (14). By combining D β sense primer and J β anti-sense primer, i.e. D β 1-J β 1.5, D β 1-J β 1.6, D β 1-J β 2.6 and D β 2-J β 2.6, every TCR D β -J β rearrangement band could be detected (14).

Statistical Analyses

Fisher's exact probability test was used.

RESULTS AND DISCUSSION

More than 90% of the irradiated mice developed thymic lymphoma after an average latent period of 200 days in B10 mice (13). Since some mice developed thymic lymphoma as early as 8 weeks, we performed flow cytometry analyses 8 to 11 weeks after irradiation. As shown in Table 1, only 2 of 20 irradiated mice bore overt thymic lymphoma, and 7 showed a decreased number of thymocytes (Table 1). Normal thymocytes are composed of four subpopulations, CD4⁻CD8⁻ double negative (DN), CD4⁺CD8⁺ double positive (DP), CD4⁺CD8⁻ single positive (SP), and CD4⁻CD8⁺ SP cells. DN cells are the most immature cells

TABLE 1
Effect of Radiation on the Regeneration of Thymocytes

Weeks after irradiation	Cell count ^a ($\times 10^6$)	CD4/CD8 staining profile	Elevation in ROS ^b
8	4.2* ^c	Not done	no
8	0.5*	Not done	yes (1.7)
8	1.2	Not done	no
9	0.6*	normal	yes (1.7)
9	1.9	normal	yes (1.5)
9	6* ^c	DP/CD8	no
9	0.14*	CD8	yes (2.5)
9	2.5*	DP/CD8	no
9	0.16*	DP/CD8	yes (3.3)
9	1.7	DP/CD8	yes (1.5)
10	1.3	normal	no
10	1.2	DP	yes (1.3)
10	0.58*	DP/CD8	yes (1.9)
10	2.4	CD8	yes (1.7)
10	2.3	CD8	yes (2.1)
11 (no. 6) ^d	2.3	CDP/CD8	yes (1.5)
11 (no. 7) ^d	1.7	normal	no
11 (no. 8) ^d	0.53*	DP/CD8	yes (2.2)
11 (no. 9) ^d	1.8	DP	yes (1.4)
11 (no. 10) ^d	0.55*	DP	yes (1.6)

^a Seventeen littermates served as controls. Thymocyte counts in control mice were $1.65 \pm 0.43 \times 10^6$ ($n = 17$). *Those cell counts were more or less than 2 SD away from the thymocyte counts in the control mice.

^b Elevation of ROS was determined if the mean fluorescence intensity of DHR staining exceeded more than 3 SD of the control mice. The elevation in ROS was measured as the ratio between the mean fluorescence intensity of each experimental mouse and the average mean fluorescence intensity of the control mice.

^c These two mice were diagnosed as having thymic lymphoma.

^d These five mice were tested for TCR β gene rearrangement. Numbers in parentheses are numbers of the mice in the experiment in Fig. 2.

that differentiate into DP cells through intermediate CD8 SP cells. Only a small fraction of DP cells differentiate into either CD4 or CD8 SP cells after so-called thymic selection, and these SP cells immigrate to peripheral lymphoid organs (15, 16). Figure 1A shows the representative staining profiles of these four thymocyte subpopulations in normal thymus. In contrast to control mice, 13 of 17 irradiated mice showed aberrant staining profiles (Table 1), in which CD8 SP and/or DP cells increased independent of thymocyte cell numbers (Fig. 1B-D).

Next we measured the levels of ROS in mitochondria in thymocytes by DHR staining. In each experiment, the reference level of ROS in mitochondria was determined in three to five control mice, and the mean fluorescence intensity of DHR staining was calculated. We judged a mouse to have elevated levels of ROS if the mean fluorescence intensity of DHR staining was more than the mean plus 3 SD of control mice. The levels of ROS in normal mice were fairly constant from mouse to mouse (Fig. 1E). In contrast, 14 of 20 irradiated mice showed an increase in ROS in mitochondria (Table 1). Representative DHR staining profiles are shown in the lower panel of Fig. 1. The difference in DHR staining levels was not due solely to the difference