INT. J. RADIAT. BIOL 2003, VOL. 79, NO. 2, 129–136

Taylor & Francis
healthsciences

Radiation dose-dependent increases in inflammatory response markers in A-bomb survivors

T. HAYASHI†*, Y. KUSUNOKI†, M. HAKODA‡, Y. MORISHITA†, Y. KUBO†, M. MAKI†, F. KASAGI§, K. KODAMA‡, D. G. MACPHEE† and S. KYOIZUMI†

(Received 17 May 2002; accepted 4 September 2002)

Abstract.
Purpose: The well-documented increases in malignant tumours in the A-bomb survivors have recently been supplemented by reports that non-cancer diseases, including cardiovascular disease, may also have increased in incidence with increasing radiation dose. Given that low-level inflammatory responses are widely accepted as a significant risk factor for such diseases, we undertook a detailed investigation of the long-term effects of ionizing radiation on the levels of the inflammatory markers C-reactive protein (CRP) and interleukin 6 (IL-6) in A-bomb survivors.
Materials and methods: Blood samples were taken from 453 participants in a long-term epidemiological cohort of A-bomb survivors. Plasma levels of CRP and IL-6 were measured using standard antibody-mediated procedures. Relationships between CRP or IL-6 levels and radiation dose were then investigated by multivariate regression analysis. Blood lymphocytes from each individual were used for immunophenotyping by flow cytometry with murine monoclonal antibodies to CD3, CD4 and CD8.
Results: CRP levels were significantly increased by about 31% Gy^{-1} of estimated A-bomb radiation ($p=0.0001$). Higher CRP levels also correlated with age, male gender, body mass index and a history of myocardial infarction. After adjustments for these factors, CRP levels still appeared to have increased significantly with increasing radiation dose (about 28% increase at 1 Gy, $p=0.0002$). IL-6 levels also appeared to have increased with radiation dose by 9.3% at 1 Gy ($p=0.0003$) and after multiple adjustments by 9.8% at 1 Gy ($p=0.0007$). The elevated CRP and IL-6 levels were associated with decreases in the percentages of $CD4^+$ helper T-cells in peripheral blood lymphocyte populations.
Conclusions: Our results appear to indicate that exposure to A-bomb radiation has caused significant increases in inflammatory activity that are still demonstrable in the blood of A-bomb survivors and which may lead to increased risks of cardiovascular disease and other non-cancer diseases.

1. Introduction

The results of epidemiological studies conducted since the establishment of the Atomic Bomb Casualty Commission–Radiation Effects Research Foundation (ABCC-RERF) in 1947 have clearly demonstrated several important long-term effects of A-bomb radiation in humans (Shigematsu 1998). The most prominent effects are radiation dose-dependent increases in the incidence of, and mortality due to, malignant tumours (Pierce *et al.* 1991, 1996, Shimizu *et al.* 1991). More recently, Kodama *et al.* (1996) and Shimizu *et al.* (1999) have detected significant increases in cardiovascular disease (CVD) among A-bomb survivors. Possible reasons for radiation-induced increases in CVD incidence and mortality are not yet known.

There is an emerging view that inflammatory processes are important in the development of atherosclerosis (Ross 1999). The pathological evidence is strong, and recent large-scale epidemiological studies suggest that even quite small increases in C-reactive protein (CRP) levels, which provide an accurate indication of levels of inflammation, may be construed as an important risk factor in inflammation which may be useful in predicting susceptibility to myocardial infarction (MI), stroke or peripheral arterial disease (Ridker *et al.* 1997, 2000, 2001, Koenig *et al.* 1999, Danesh *et al.* 2000, Mendall *et al.* 2000). It is therefore interesting to note that erythrocyte sedimentation rates, white blood cell counts and sialic acid levels, all of which tend to increase during inflammatory responses, also appear to increase with radiation dose in A-bomb survivors when measured 40 or so years after the bombing (Neriishi *et al.* 2001).

Our major aim was to obtain accurate measurements of the current plasma CRP levels of A-bomb survivors, mainly for use as an indicator of their inflammation status half a century or so after the bombing. We also sought to measure the current plasma levels of interleukin 6 (IL-6), a pleiotropic cytokine which has a wide range of effects on humoral and cellular responses and acts as a primary inducer of the CRP produced by the liver in response to a number of pro-inflammatory stimulants (Kishimoto 1989, Heinrich *et al.* 1990). Increased levels of IL-6, even within the healthy reference range, have been shown to mimic increased levels of CRP in being associated with an increased risk of CVD in a prospective epidemiological study (Ridker *et al.* 2000).

*Author for correspondence; e-mail: tomo@rerf.or.jp
Departments of †Radiobiology, ‡Clinical Studies and §Statistics, Radiation Effects Research Foundation, 5-2 Hijiyama Park, Minami Ward, Hiroshima 732-0815 Japan.

International Journal of Radiation Biology ISSN 0955-3002 print/ISSN 1362-3095 online © 2003 Taylor & Francis Ltd
http://www.tandf.co.uk/journals
DOI: 10.1080/0955300021000038662

Importantly for our purposes, others have shown that whole-body irradiation led to increases in IL-6 levels in both human and animal systems (Herodin *et al.* 1992, Girinsky *et al.* 1994, Haveman *et al.* 1998). Here we report the detection of radiation dose-related increases in both CRP and IL-6 in plasma samples taken from 453 A-bomb survivors approximately 50 years after the Hiroshima bombing and discuss the possible significance of these increases for CVD. Furthermore, since immunological studies have revealed long-term impairment of $CD4^+$ helper T-cell immunity (Kusunoki *et al.* 1998) and an association between a decreased proportion of $CD4^+$ T-cells and a history of MI in A-bomb survivors (Kusunoki *et al.* 1999), we also discuss associations between impaired helper T-cell immunity and the increased levels of IL-6 and CRP in the survivors.

2. Materials and methods

2.1. *Subjects*

Subjects were randomly selected from participants in an epidemiological follow-up study of A-bomb survivors (adult health study; AHS) that has been running since 1958. Details of this study have been published by Wong *et al.* (1993). Each participant was invited to attend for clinical examination at RERF every 2 years. For the present study, blood samples were obtained from the clinical study subjects in Hiroshima City between March 1995 and April 1997. We obtained institutional approval from the human investigation committee and informed consent from participants. Participants whose blood samples were drawn after they had been diagnosed with cancer or an inflammatory disease such as current cold, chronic bronchitis and collagen diseases including rheumatoid arthritis were excluded from this study. Estimated bone marrow doses were based on the 1986 Dosimetry System known as DS86; basically, it involves calculating a free-in-air radiation dose estimate for the subject's reported location and then adjusting the value obtained to reflect shielding information (Roesch 1987). The unit of absorbed ionizing radiation for both gamma-rays and neutrons is the gray (Gy). Four hundred and fifty subjects in Hiroshima were selected from radiation-exposed (high dose and medium-low dose) and non-exposed groups so that the age and gender distributions were similar in the three groups. One hundred and seven subjects were assigned DS86 doses more than 1.5 Gy (high dose group), 164 doses of between 0.005 and 1.5 Gy (medium-low dose group) and 182 doses of less than 0.005 Gy (control group). Background characteristics of these three dose groups are summarized in table 1.

2.2. *Laboratory methods*

Plasma CRP levels were measured using a CRP-Latex kit (Nissui Pharmaceutical Co. Ltd, Tokyo, Japan) containing anti-CRP monoclonal antibodies. The detection limit of this particular kit was $0.01\,\mathrm{mg\,dl^{-1}}$. Since the measurements were done with an Hitachi 7170 auto-analyser (Tokyo, Japan), a robotic system used for assaying many routine clinical biochemical parameters, the results tend to be highly reproducible and the coefficient of variation (CV) at the CRP concentration of $0.1\,\mathrm{mg\,dl^{-1}}$ was 1.5%. IL-6 levels were determined using an exceptionally sensitive enzyme-linked immunosorbent assay kit (Quantikine HS, R&D systems, Minneapolis, MN, USA) whose minimum detectable concentration was said to be $0.09\,\mathrm{pg\,ml^{-1}}$.

Total cholesterol (TC), high-density lipoprotein (HDL) cholesterol and triglycerides (TG) were assayed on the auto-analyser. Immunophenotyping of blood lymphocytes was performed with peripheral blood mononuclear cell fractions using a FACScan flow cytometer (Beckton Dickinson, San Jose, CA, USA) and fluorescein- and phycoerythrin-labelled murine monoclonal IgG antibodies to CD3, CD4 and CD8 (Beckton Dickinson) as described by Kusunoki *et al.* (1998).

2.3. *Statistical analysis*

We assessed possible differences in the background characteristics of the three radiation dose groups using trend tests. Based on applied regression analysis with dependent variable $= \alpha + \beta \times$ dose for continuous variables (age, clinical data, CRP, IL-6 and lymphocyte subset percentage), and logistic analysis with logit function $= \alpha + \beta \times$ dose for dichotomous variables (gender). In this analysis, 'dose' refers to the mean dose (0, 0.66 or 2.30 Gy) for each of the dose groups (control, low-medium or high dose). The significance level of coefficient β was expressed as trend test p value. The primary aim of this study was to determine whether A-bomb radiation caused any significant changes to plasma CRP or IL-6 levels in those who survived for five decades or more after exposure. Thus, although the study subjects were assigned to the three main dose groups in order to make assessments of similarities and differences in their basic characteristics, we made use of *individual* doses when it came to examining the data for possible associations of plasma CRP (and IL-6) levels with dose. A logarithmic transformation was applied to

Table 1. Characteristics of the study subjects.

	Radiation dose groups			
	Non-exposed ($n=182$)	0.005–1.5 Gy ($n=164$)	>1.5 Gy ($n=107$)	p for trend[a]
Radiation dose (Gy)*	0	0.66±0.45	2.30±0.72	
Age (years)*	68.5±10.5	68.9±10.8	67.3±10.4	0.442
Gender (F/M)†	98/84	93/71	58/49	0.88
Smoking†, *n* (%)	44 (24.2)	36 (22.0)	27 (25.2)	0.92
Body-mass index (kg m^{-2})*	22.8±3.3	22.7±3.4	22.5±3.4	0.75
Total cholesterol (mg dl^{-1})*	213.3±35.3	210.0±36.9	203.4±34.5	0.027
HDL cholesterol (mg dl^{-1})*	53.2±14.9	50.3±14.1	48.7±14.0	0.007
Triglycerides (mg dl^{-1})	144.3±84.7	147.7±86.6	160.0±115.7	0.187
Systolic blood pressure (mmHg)*	135.8±21.8	135.0±23.9	135.1±21.9	0.79
History of myrocardial infarction, *n* (%)†	3 (1.6)	5 (3.0)	3 (2.8)	0.479
CRP (mg dl^{-1})‡	0.050 (0.041–0.061)	0.059 (0.048–0.072)	0.093 (0.074–0.116)	<0.001
IL-6 (pg ml^{-1})‡	1.47 (1.35–1.59)	1.53 (1.40–1.68)	1.85 (1.65–2.07)	0.003
CD3$^+$ (%)‡	59.5 (58.0–61.0)	57.0 (55.3–58.7)	56.5 (54.3–58.6)	0.012
CD3$^+$CD4$^+$ (%)‡	47.2 (45.8–48.50)	45.8 (44.4–47.3)	44.6 (42.7–46.4)	0.02
CD3$^+$CD8$^+$ (%)‡	12.1 (11.28–12.96)	11.6 (10.5–12.6)	12.0 (11.0–13.1)	0.80

[a]Comparison of characteristics across three radiation dose groups.
Data are *plus–minus values are mean±SD; †number of participants; ‡mean (95% confidence interval).
HDL, high-density lipoprotein.
Trends were tested by regression analysis for continuous values (age, clinical data, CRP, IL-6 and lymphocyte subset percentage) and for dichotomous values (gender).

the dependent variables representing CRP and IL-6 levels to obtain a distribution as close to normal as possible. The association of a factor (v) with log-transformed CRP or IL-6 levels was analysed by applying the regression model in a three-ordered manner:

- Univariate model: $\log(\mathrm{CRP})=\alpha+\beta_1\times v$.
- Multivariate model with adjustment for sex and age: $\log(\mathrm{CRP})=\alpha+\beta_1\times v+\beta_2\mathrm{sex}+\beta_3\mathrm{age}$.
- Multivariate model with adjustment for sex, age, and other background factors (X): $\log(\mathrm{CRP})=\alpha+\beta_1\times v+\beta_2\mathrm{sex}+\beta_3\mathrm{age}+\beta_4 X$.

Accordingly, the increased rate of CRP for 1 unit of factor v was calculated as $e^{\beta_1\times\mathrm{unit}}$ and its per cent increment was $100(e^{\beta_1\times\mathrm{unit}}-1)$. The associations between the levels of CRP or IL-6 and CD4$^+$ T-cell proportion were also analysed in a three-ordered manner. All statistical analyses were carried out using the SAS program (SAS Institute, Inc., Cary, NC, USA).

3. Results

We examined samples from 249 women and 204 men with a mean age of 68.3±10.6 years (table 1). There were no significant differences in the mean age, percentage of smokers, mean of body-mass index (BMI), triglycerides and systolic blood pressure among the three radiation dose groups, while the means of total and HDL cholesterol levels appeared to decrease slightly with increasing radiation dose.

The mean levels of CRP tended to increase with radiation dose (table 1). Univariate regression analysis showed that there was a significant positive correlation between plasma CRP levels and radiation dose ($p=0.0001$; table 2). Age, BMI, triglycerides, HDL cholesterol, systolic blood pressure and a history of MI also had a significant effect on CRP levels, but there appeared to be no gender or smoking effect (table 2). Disease histories other than MI did not show any significant effects on CRP levels. All of these factors were employed as adjustment factors in the multivariate regression analysis of the relationship between CRP levels and radiation dose. Even with these adjustments, plasma CRP levels still appeared to be significantly and positively correlated with the radiation dose, to the extent of about 28% increase per Gy of radiation ($p=0.0002$; table 2).

Next, we analysed the levels of IL-6, a major inducer of CRP, in the same plasma samples used for CRP measurements. Mean levels of IL-6 also

Table 2. Regression analysis for association with CRP levels.

	Univariate		Multivariate (adjusted for age and gender)		Multivariate (fully adjusted[c])	
Factor[a]	Per cent increment per unit[b]	*p*	Per cent increment per unit[b]	*p*	Per cent increment per unit[b]	*p*
Age	20.1 (7.2–33.2)	0.002			21.6 (8.1–35.3)	0.002
Gender	−3.8 (−36.5–21.1)	0.79			−10.6 (−17.4–48.0)	0.50
Smoking	−61.2 (−362.0–244.0)	0.69	33.6 (−298.2–371.0)	0.84	119.4 (−200.6–444.6)	0.47
BMI	9.9 (5.6–14.4)	0.0001	10.6 (6.3–15.1)	0.0001	8.2 (3.8–12.7)	0.0002
TC	−1.5 (−5.3–2.4)	0.45	−0.9 (−4.8–3.1)	0.65	0.9 (−3.2–5.0)	0.68
HDL	−2.2 (−3.1–−1.3)	0.0001	−2.3 (−3.2–−1.3)	0.0001	−1.7 (−2.7–−0.6)	0.003
TG	1.8 (0.3–3.3)	0.016	2.1 (0.7–3.6)	0.005	0.2 (−1.5–1.8)	0.84
SBP	9.3 (3.3–15.4)	0.003	7.2 (1.0–13.6)	0.02	4.1 (−2.1–10.3)	0.20
MI	185.6 (18.3–589.5)	0.02	163.1 (9.3–533.6)	0.03	135.8 (2.4–443.1)	0.04
Radiation dose	30.7 (14.2–49.6)	0.0001	32.9 (16.2–51.9)	0.0001	28.1 (12.4–46.0)	0.0002

[a]BMI, body mass index; TC, total cholesterol; HDL, high-density lipoprotein cholesterol; TG, triglycerides; SBP, systolic blood pressure; MI, history of myocardial infarction.

[b]Numbers in parentheses denote the 95% confidence interval and the unit of age is 10 years; gender female = 0 and male = 1; smoking one cigarette package; BMI = 1 kg m^{-2}; TC = 10 mg dl^{-1}; TG = 10 mg dl^{-1}; HDL = 10 mg dl^{-1}; SBP = 10 mmHg; MI without history of MI = 0 and with history of Mi = 1; radiation dose = 1 Gy.

[c]Multiple adjustment was performed for all other factors.

tended to increase with radiation dose (table 1). Univariate regression analysis indicated that there was about a 9% increase in IL-6 levels at 1 Gy of radiation dose ($p = 0.003$; table 3). Age, total cholesterol, systolic blood pressure and a history of MI had a significant effect on IL-6 levels, and smoking also had a significant effect in this situation ($p = 0.01$). After adjustments were made for the various factors, there was still evidence of a significant positive correlation between IL-6 levels and radiation dose, with an increase of approximately 10% in the IL-6 level per Gy of radiation ($p = 0.0001$).

Unsurprisingly, the CRP and IL-6 levels appeared to be positively correlated when all subjects were assessed (correlation coefficient, $r = 0.42$, $p = 0.0001$). This correlation seemed to be stronger in non-exposed controls ($r = 0.534$, $p < 0.0001$) than in the exposed subjects (combined subjects of high dose and medium-low dose group) ($r = 0.430$, $p < 0.0001$), although the difference was not statistically significant. The increases in CRP levels with radiation dose in A-bomb survivors may therefore be a function of the levels of increase in IL-6 levels that we were observing, and even although the association between CRP level and radiation dose turned out to be considerably weaker after adjusting for IL-6 levels, it nonetheless remained significant, with adjustment causing the CRP levels at 1 Gy to fall by less than 50% (from 28.1%, $p = 0.0002$, to 17.7%, $p = 0.0098$).

As shown in table 1 and as noted in Kusunoki *et al.* (1998), the proportion of $CD3^+$ T-cells tends to decrease with radiation dose. This decrease in $CD3^+$ T-cells was due to a decrease in $CD3^+CD4^+$ T-cells since their $^+$ counterpart $CD3^+CD8^+$ T-cells did not appear to change in proportion with radiation dose. Regression analysis revealed that there had been a 2.6% decrease in $CD4^+$ T-cell proportion at 1 Gy after adjustment for age and gender; both adjustment factors appeared to have significant effects on $CD4^+$ T-cell proportion ($p = 0.02$). Since $CD4^+$ T-cells are known to play important roles in defending against infectious agents which are the most common cause of inflammation, correlations between the levels of CRP or IL-6 and $CD4^+$ T-cell proportion were analysed. As shown in figure 1, the levels of both CRP and IL-6 appeared to increase with decreasing $CD4^+$ T-cell proportion. Although there seemed to be some threshold value of $CD4^+$

Table 3. Regression analysis for association with IL-6 levels.

	Univariate		Multivariate (adjusted for age and gender)		Multivariate (fully adjusted[c])	
Factor[a]	Per cent increment per unit[b]	*p*	Per cent increment per unit[b]	*p*	Per cent increment per unit[b]	*p*
Age	21.2 (15.9–26.5)	0.0001			20.0 (14.4–25.7)	0.0001
Gender	11.0 (−0.1–20.9)	0.05			2.4 (−10.0–13.5)	0.69
Smoking	115.8 (−14.8–247.4)	0.08	174.8 (40.2–310.6)	0.01	163.8 (31.0–297.6)	0.02
BMI	0.6 (−1.2–2.4)	0.52	1.3 (−0.4–3.0)	0.12	1.0 (−0.8–2.7)	0.27
TC	−3.5 (−5.1–−1.9)	0.0001	−2.5 (−4.1–−0.9)	0.002	−2.5 (−4.2–−0.8)	0.005
HDL	−0.3 (−0.7–−0.1)	0.13	−0.3 (−0.7–0.1)	0.16	−1.7 (−2.7–0.9)	0.57
TG	−0.1 (−0.7–0.5)	0.78	0.2 (−0.4–0.8)	0.59	0.2 (−0.5–1.8)	0.84
SBP	7.0 (4.5–9.6)	0.0001	4.3 (1.7–6.8)	0.001	3.7 (1.1–6.3)	0.005
MI	62.5 (11.2–137.3)	0.01	43.5 (0.5–104.7)	0.04	47.2 (4.4–107.6)	0.03
Radiation dose	9.3 (3.1–15.9)	0.003	32.9 (16.2–51.9)	0.0001	9.8 (4.1–15.9)	0.0007

See notes to table 2.

T-cell proportion for the change in the levels of CRP and IL-6, statistical evaluation for any such threshold was difficult. Thus, regression analysis was performed. As shown in table 4, both CRP and IL-6 levels were negatively associated with $CD4^+$ T-cell proportion. This negative association was still highly significant after adjustment for the various factors that appeared to be significantly associated with CRP or IL-6 levels (tables 2 and 3).

4. Discussion

Our present study shows that plasma CRP levels, which are often used as a sensitive marker of inflammatory activity, appear to increase with radiation dose in a cohort of A-bomb survivors. Since the correlation between CRP levels and radiation dose remained significant even after adjustments for other factors that are either known or widely assumed to cause plasma CRP levels to change, it could reasonably be inferred that A-bomb radiation exposure is likely to have been an important factor in the increases in CRP levels detected in A-bomb survivors up to half a century after the bombing. In accordance with the established fact that IL-6 acts as a primary inducer of CRP production in the liver (Kishimoto 1989, Heinrich *et al.* 1990), we found that CRP levels correlated very well with the increase in IL-6 levels in our present study of A-bomb survivors. Also, given that IL-6 is rapidly produced in response to various inflammatory stimuli (Heinrich *et al.* 1990), our present results with elevated IL-6 levels strongly support our conclusion that inflammatory processes are active (albeit at quite low levels) in radiation-exposed subjects.

It is not known whether plasma CRP and IL-6 levels reached their present levels soon after the bombing (i.e. in 1945 or shortly thereafter) or were steadily rising during the years before 1995–97, which is when we obtained the samples for the tests reported here. However, the results of biennial medical examinations of the A-bomb survivors in earlier clinical examination cycles (in the 1960s) indicate that there were already significant dose-related changes in white blood cell counts and erythrocyte sedimentation rates (Sawada *et al.* 1986). Since the levels of both markers are known to rise in response to a variety of inflammatory stimuli (Heinrich *et al.* 1990), it seemed reasonable to assume that low levels of inflammation may well have been discernible in the survivors for very long periods, up to and including the sampling period in 1995–97.

Since the opinion of several recent investigators is that even quite low levels of inflammation may well constitute an independent risk factor for CVD (Ridker *et al.* 1997, 2001, Koenig *et al.* 1999, Danesh *et al.* 2000, Mendall *et al.* 2000), it would be of great interest to find out whether the dose-dependent

increases in CRP levels that we observed in A-bomb survivors are also associated with an increased risk of CVD. Moreover, although the sources of the low levels of inflammation that seem to be associated with CVD risk in apparently healthy individuals have not yet been determined, a recent report suggests that increased levels of IL-6 may also be predictive of an increased risk of MI (Ridker *et al.* 2000). Thus our finding of correlated increases in CRP and IL-6 levels in radiation-exposed A-bomb survivors could well be indicative of low levels of persistent inflammation that could presage an increased risk of CVD for the survivors concerned. Clearly, a prospective study will be required to test this hypothesis directly.

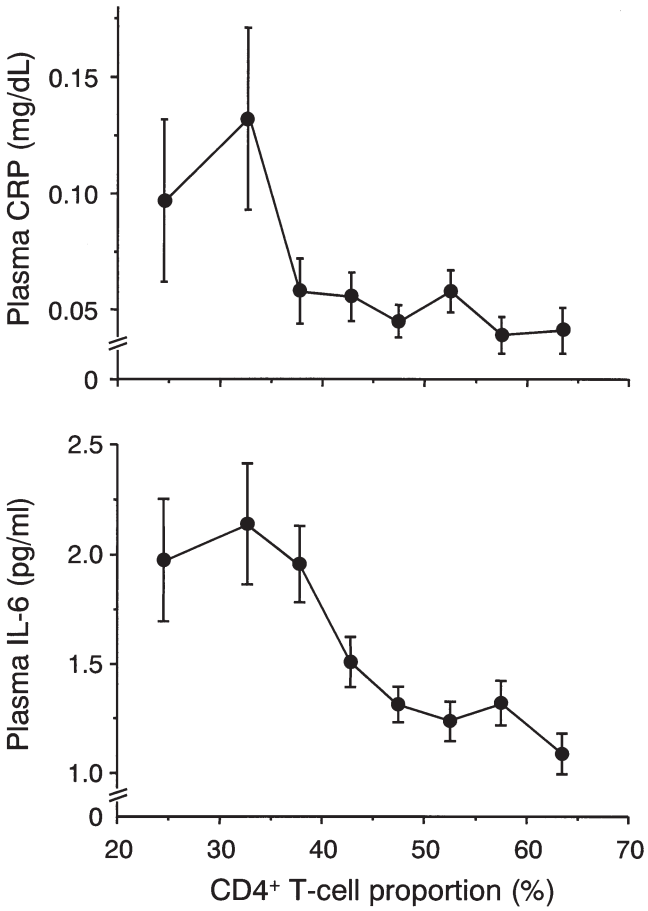

Figure 1. Negative association of the $CD4^+$ T-cell proportions with the plasma CRP and IL-6 levels. Each plot shows the mean and standard error of plasma levels of CRP (upper) and IL-6 (lower) in each 5% category of $CD4^+$ T-cell proportion.

The mechanisms underlying the persistently increased inflammatory responses that we detected in A-bomb survivors have not yet been determined. Increases in blood levels of IL-6 after total-body irradiation have been observed in patients who had recently undergone whole-body irradiation for bone marrow transplantation (Girinsky *et al.* 1994). These elevated IL-6 levels were found to have returned to baseline within 24 h, however, indicating that the more immediate effects of whole-body irradiation on this particular manifestation of an inflammatory response do not persist for terribly long periods in these patients. Comparable results were obtained in animal (rat) experiments in which the increased levels of IL-6 that occurred in response to whole-body irradiation had also dropped back to baseline levels within 24 h (Haveman *et al.* 1998). However, long-term experiments have not been performed to test whether IL-6 levels could increase again after their initial return to baseline levels in both humans and animals.

It is of course also possible that certain of the less direct effects of whole-body irradiation on humans cause—or contribute to—the persistently increased (but still relatively mild) symptoms of inflammatory activity that we see in A-bomb survivors. It may therefore be worth noting that we have previously reported and presented here a dose-dependently decreased representation of $CD4^+$ T-cells in A-bomb survivors (Kusunoki *et al.* 1998). Our observation that the CRP and IL-6 levels measured in this study were negatively correlated with the proportions of $CD4^+$ T-cells in A-bomb survivors may therefore be of some considerable interest. Since $CD4^+$ T-cells

Table 4. Regression analysis for association of CRP and IL-6 levels with $CD4^+$ T-cell proportion.

	Effect of 5% increase in $CD4^+$ T-cell proportion			
Adjustment	Per cent change in CRP[a]	*p*	Per cent change in IL-6[a]	*p*
Univariate	−11.4 (−17.9 to −4.8)	0.0008	−8.7 (−11.5 to −5.9)	0.0001
Multivariate (adjusted for age and gender)	−9.0 (−15.9 to −1.9)	0.01	−5.3 (−8.1 to −2.5)	0.0002
Multivariate (fully adjusted[b])	−7.0 (−13.7 to −0.2)	0.04	−5.0 (−7.7 to −2.2)	0.0007

[a]Log-transformed values of CRP and IL-6 as dependent variables were used in the linear regression analyses. Numbers in parentheses denote the 95% confidence interval.

[b]Adjusted for age, gender, smoking, body mass index, total cholesterol, high-density lipoprotein cholesterol, systolic blood pressure and radiation dose.

have an important role in defending the body against infectious agents, it is not unrealistic to suggest that A-bomb survivors with decreased $CD4^+$ T-cell numbers may be somewhat more susceptible to infections of various sorts than their non-exposed counterparts. Consistent with this expectation is the fact that A-bomb survivors appear to display a radiation dose-related increase in mortality due to pneumonia (Shimizu *et al.* 1999). Viral infections may also occur at elevated frequencies in A-bomb survivors, as suggested by a finding that the prevalence of hepatitis B surface antigen appears to increase with increasing A-bomb irradiation (Neriishi *et al.* 1995). One may also envisage asymptomatic infectious events being of some relevance to the increased inflammatory responses observed in our present study.

Clearly, we are still obtaining new and unexpected information about the long-term effects of ionizing radiation on humans. Our present study establishes that one of the more sensitive markers of inflammatory status, an individual's CRP levels, still seem to be dose-dependently elevated in the A-bomb survivors more than half a century after the bombing of Hiroshima. We also found that there was a correlation between increased CRP levels and contemporaneous plasma levels of IL-6. Although we can not say much about the significance of increased levels of CRP and IL-6 in diseases of A-bomb survivors as yet, we believe there is a good chance that such inflammatory reactions we have detected will prove to be associated with an increase in CVD risk, in much the same way as they were seen to be in numerous epidemiological studies of unirradiated and otherwise seemingly normal individuals (Ridker *et al.* 1997, 2001, Koenig *et al.* 1999, Danesh *et al.* 2000, Mendall *et al.* 2000). Thus our present results appear to imply that further studies of radiation-associated inflammatory responses are likely to provide us with important new information about one or more of the less well-documented, but nonetheless far from trivial, long-term effects of ionizing radiations in humans.

Acknowledgements

This paper was based on research performed at the Radiation Effects Research Foundation (RERF), Hiroshima and Nagasaki, Japan. RERF is a private, non-profit foundation funded equally by the Japanese Ministry of Health, Labour and Welfare and the US Department of Energy through the National Academy of Sciences.

References

Danesh, J., Whincup, P., Walker, M., Lennon, L., Thomson, A., Appleby, P., Gallimore, J. R. and Pepys, M. B., 2000, Low grade inflammation and coronary heart disease: prospective study and updated meta-analyses. *British Medical Journal*, **321,** 199–204.

Girinsky, T. A., Pallardy, M., Comoy, E., Benassi, T., Roger, R., Ganem, G., Cosset, J. M., Socie, G. and Magdelenat, H., 1994, Peripheral blood corticotropin-releasing factor, adrenocorticotropic hormone and cytokine (interleukin beta, interleukin 6, tumor necrosis factor alpha) levels after high- and low-dose total-body irradiation in humans. *Radiation Research*, **139,** 360–363.

Haveman, J., Geerdink, A. G. and Rodermond, H. M., 1998, TNF, IL-1 and IL-6 in circulating blood after total-body and localized irradiation in rats. *Oncology Reports*, **5,** 679–683.

Heinrich, P. C., Castell, J. V. and Andus, T., 1990, Interleukin-6 and the acute phase response. *Biochemical Journal*, **265,** 621–636.

Herodin, F., Mestries, J. C., Janodet, D., Martin, S., Mathieu, J., Gascon, M. P., Pernin, M. O. and Ythier, A., 1992, Recombinant glycosylated human interleukin-6 accelerates peripheral blood platelet count recovery in radiation-induced bone marrow depression in baboons. *Blood*, **80,** 688–695.

Kishimoto, T., 1989, The biology of interleukin-6. *Blood*, **74,** 1–10.

Kodama, K., Mabuchi, K. and Shigematsu, I., 1996, A long-term cohort study of the atomic-bomb survivors. *Journal of Epidemiology*, **6,** S95–105.

Koenig, W., Sund, M., Frohlich, M., Fischer, H. G., Lowel, H., Doring, A., Hutchinson, W. L. and Pepys, M. B., 1999, C-Reactive protein, a sensitive marker of inflammation, predicts future risk of coronary heart disease in initially healthy middle-aged men: results from the MONICA (Monitoring Trends and Determinants in Cardiovascular Disease) Augsburg Cohort Study, 1984 to 1992. *Circulation*, **99,** 237–242.

Kusunoki, Y., Kyoizumi, S., Hirai, Y., Suzuki, T., Nakashima, E., Kodama, K. and Seyama, T., 1998, Flow cytometry measurements of subsets of T, B and NK cells in peripheral blood lymphocytes of atomic bomb survivors. *Radiation Research*, **150,** 227–236.

Kusunoki, Y., Kyoizumi, S., Yamaoka, M., Kasagi, F., Kodama, K. and Seyama, T., 1999, Decreased proportion of CD4 T cells in the blood of atomic bomb survivors with myocardial infarction. *Radiation Research*, **152,** 539–543; erratum **154,**119.

Mendall, M. A., Strachan, D. P., Butland, B. K., Ballam, L., Morris, J., Sweetnam, P. M. and Elwood, P. C., 2000, C-reactive protein: relation to total mortality, cardiovascular mortality and cardiovascular risk factors in men. *European Heart Journal*, **21,** 1584–1590.

Neriishi, K., Akiba, S., Amano, T., Ogino, T. and Kodama, K., 1995, Prevalence of hepatitis B surface antigen, hepatitis B e antigen and antibody, and antigen subtypes in atomic bomb survivors. *Radiation Research*, **144,** 215–221.

Neriishi, K., Nakashima, E. and Delongchamp, R. R., 2001, Persistent subclinical inflammation among A-bomb survivors. *International Journal of Radiation Biology*, **77,** 475–482.

Pierce, D. A., Shimizu, Y., Preston, D. L., Vaeth, M. and Mabuchi, K., 1996, Studies of the mortality of atomic

bomb survivors. Report 12, Part I. Cancer: 1950–1990. *Radiation Research*, **146,** 1–27.

Pierce, D. A., Vaeth, M. and Preston, D. L., 1991, Analysis of time and age patterns in cancer risk for A-bomb survivors. *Radiation Research*, **126,** 171–186.

Ridker, P. M., 2001, High-sensitivity C-reactive protein: potential adjunct for global risk assessment in the primary prevention of cardiovascular disease. *Circulation*, **103,** 1813–1818.

Ridker, P. M., Cushman, M., Stampfer, M. J., Tracy, R. P. and Hennekens, C. H., 1997, Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. *New England Journal of Medicine*, **336,** 973–979.

Ridker, P. M., Rifai, N., Stampfer, M. J. and Hennekens, C. H., 2000, Plasma concentration of interleukin-6 and the risk of future myocardial infarction among apparently healthy men. *Circulation*, **101,** 1767–1772.

Ridker, P. M., Stampfer, M. J. and Rifai, N., 2001, Novel risk factors for systemic atherosclerosis: a comparison of C-reactive protein, fibrinogen, homocysteine, lipoprotein(a), and standard cholesterol screening as predictors of peripheral arterial disease. *Journal of the American Medical Association*, **285,** 2481–2485.

Roesch, W. C., 1987, *US–Japan Joint Reassessment of Atomic Bomb Radiation Dosimetry in Hiroshima and Nagasaki* (Hiroshima: Radiation Effects Research Foundation).

Ross, R., 1999, Atherosclerosis — an inflammatory disease. *New England Journal of Medicine*, **340,** 115–126.

Sawada, H., Kodama, K., Shimizu, Y. and Kato, H., 1986, Adult health study report 6. Results of six examination cycles, 1968–1980, Hiroshima and Nagasaki. *Radiation Effects Research Foundation Technical Reports*, 3–86.

Shigematsu, I., 1998, Greetings: 50 years of Atomic Bomb Casualty Commission–Radiation Effects Research Foundation studies. *Proceedings of the National Academy of Sciences of the United States of America*, **95,** 5424–5425.

Shimizu, Y., Kato, H. and Schull, W. J., 1991, Risk of cancer among atomic bomb survivors. *Journal of Radiation Research (Tokyo)*, **32 (Suppl. 2),** 54–63.

Shimizu, Y., Pierce, D. A., Preston, D. L. and Mabuchi, K., 1999, Studies of the mortality of atomic bomb survivors. Report 12, Part II. Noncancer mortality: 1950–1990. *Radiation Research*, **152,** 374–389.

Wong, F. L., Yamada, M., Sasaki, H., Kodama, K., Akiba, S., Shimaoka, K. and Hosoda, Y., 1993, Noncancer disease incidence in the atomic bomb survivors: 1958–1986. *Radiation Research*, **135,** 418–430.