in other rodent models. Enzyme-altered foci, which emerge before the appearance of any key indicators of liver cancer, contain a higher percentage of cells synthesizing DNA than cells in surrounding areas (Farber, 1984). This increase may be ascribed, at least in part, to decreased expression of transforming growth factor β1, a negative growth regulator in the cells comprising hepatic foci (Moser et al., 1996). In the two-stage model of skin carcinogenesis, exposure to the tumor promoter TPA elicits selective expansion of initiated keratinocytes at the expense of growth-inhibited normal keratinocytes (Karen et al., 1999). The maintenance of decreased TNFα expression in thymic macrophages contributes to the appearance of pre-lymphomic cells in radiation-induced thymic lymphomagenesis, and intraperitoneal injection of TNFα (Boniver et al., 1989) or bone marrow transplantation (Humblet et al., 1996), the latter of which induces localized TNFα production, inhibits the development of thymic lymphomas. Therefore, another element required for the expansion of initiated cells is escape from negative growth regulation imposed by surrounding normal cells. Taken together, our current knowledge suggests that both the escape from negative growth regulation and the acquisition of enhanced susceptibility to growth stimulating microenvironments are essential to elicit the expansion of initiated cells.

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Prevalence of Hepatitis B Virus Infection among Atomic Bomb Survivors

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Fujiwara, S., Sharp, G. B., Cologne, J. B., Kusumi, S., Akahoshi, M., Kodama, K., Suzuki, G. and Yoshizawa, H. Prevalence of Hepatitis B Virus Infection among Atomic Bomb Survivors. *Radiat. Res.* 159, 780–786 (2003).

The aim of this study was to determine whether the prevalence of hepatitis B virus (HBV) carriers increased with atomic bomb radiation dose, and whether radiation decreased the ability to clear HBV among the atomic bomb survivors. The study subjects were 6,121 participants in the Adult Health Study of atomic bomb survivors in Hiroshima and Nagasaki. After adjustment for age, sex, city and potential confounders, the rates of seropositivity for hepatitis B surface antigen (HBsAg), indicating current HBV infections, and anti-hepatitis B core antibody, indicating either cured or current infections, increased with radiation dose. However, no relationship was observed between radiation and anti-hepatitis B surface antibody (indicating cured infection). The proportion of persons who were unable to clear the virus, as the proportion of HBsAg-positive persons among those ever infected by HBV (positive for HBsAg or surface or core hepatitis B antibody), increased significantly with radiation dose among those receiving blood transfusions. This proportion was not related to dose among those who reported no such transfusions. The findings may suggest a lower likelihood of clearance after HBV infection among those who were more likely to have been infected with HBV as adults after atomic bomb irradiation rather than as infants or adults prior to irradiation.

INTRODUCTION

Studies of liver cancer risks in atomic bomb (A-bomb) survivors have consistently shown that exposure to A-bomb radiation significantly increases liver cancer mortality rates (*1*) and primary liver cancer incidence rates; 85% of the liver cancer is hepatocellular carcinoma (HCC) (*2, 3*). A study of a subset of A-bomb survivors who received clinical examinations showed that risks of chronic liver disease were significantly associated with radiation dose (P = 0.006) (*4*). Because infection with hepatitis B (HBV) and C (HCV) viruses is strongly associated with HCC, chronic hepatitis, and cirrhosis (*5*), several studies of atomic bomb survivors have been conducted to describe the relationship between these viruses and radiation dose (*6–9*). Although our previous report did not show a significant relationship between atomic bomb radiation and anti-HCV antibody (*9*), a study of this cohort by Neriishi *et al.* reported a 1.8-fold increase in the prevalence of hepatitis B surface antigen (HBsAg) among those with radiation exposures of 1 Sv or more (P for trend = 0.024) (*8*).

The increase in HBsAg positivity among A-bomb survivors may suggest reduced immune surveillance against HBV infection. The primary route of chronic HBV infection is by mother-to-child transmission during infancy. Generally persons who are infected with HBV as adults through blood transfusions, sexual contact or other means are able to clear the virus, with their serum HBsAg being replaced by anti-hepatitis B surface antibody (anti-HBsAb); only a small percentage of such persons become HBV carriers who are chronically infected with HBV (positive for HBsAg in serum) (*10*). Persons previously infected with HBV who were able to clear the virus are HBsAg-negative but are antibody-positive for either anti-HBsAb or anti-hepatitis B core antibody (anti-HBcAb) in serum.

The goal of the present study of atomic bomb survivors was to combine information about blood transfusions, family history of liver disease, and other factors with information about HBsAg and HBV antibody status to determine if the prevalence of HBV carriers increased with atomic bomb radiation dose, and if radiation exposure was associated with a decreased ability to clear this virus.

SUBJECTS AND METHODS

Study Subjects

The Adult Health Study (AHS) was established in 1958 to observe the health effects of radiation exposure among A-bomb survivors in Hiroshima and Nagasaki. The original AHS cohort consisted of about 10,000 radiation-exposed A-bomb survivors and about half as many nonexposed subjects who were 3–10 km from the epicenter. Subjects were selected

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from among Hiroshima and Nagasaki residents using the 1950 national census supplementary schedules, the Atomic Bomb Survivors Survey, and the 1953 daytime census (*11*). Since July 1, 1958, AHS subjects have been followed through biennial medical examinations. Included in the present study were 6,121 AHS participants in both cities who underwent medical examination from 1993–1995. The study was approved by the Research Protocol Committee and the Human Investigation Committee at RERF.

Dosimetry

Radiation dose estimates were based on the Dosimetry System 1986 (DS86) (*12*). This system estimates organ-specific radiation exposures, taking into account location at the time of the bombing and shielding by building, terrain, and surrounding body tissue. Because there is no single organ system exclusively involved in hepatitis infection, we used whole-body shielded kerma without weighting the neutron and γ-ray components by a relative biological effectiveness (RBE) factor, i.e. using an RBE of 1. Radiation doses ranged from zero for those who were well shielded or were far from the A-bomb hypocenter to a maximum of 5.6 Gy for individuals exposed at locations near the hypocenter. Doses up to 0.005 Gy for individuals are defined as zero under DS86.

Laboratory Methods and Questionnaire Survey

For all of the AHS participants, the levels of serum HBsAg, anti-HBsAb and anti-HBcAb were measured using commercial measurement kits. The same assay methods were used in Hiroshima and Nagasaki. HBsAg, a measure of current infection, and anti-HBsAb, a measure of cured infections, were measured using a reverse passive hemagglutination (PHA) test kit (Special Immunological Lab., Tokyo) and a PHA test kit (Special Immunological Lab., Tokyo), respectively. The levels of serum anti-HBcAb, a measure of either cured or current infection, were determined using a PHA test kit (Red-Cross Hospital, Hiroshima). In screening tests, individuals were diagnosed as having antibody or antigen when agglutination was found in a serum diluted 2^2 (fourfold) for HBsAg, 2^3 (eightfold) for anti-HBsAb, and 2^4 (16-fold) for anti-HBcAb according to the manufacturer's instructions. For individuals having a positive response to the HBsAg or any of the HBV antibodies, the magnitude of response was measured by serial twofold dilution methods. The index number at the maximum dilution in which a positive reaction was found was defined as the titer of each antibody or antigen. We defined a titer of 2^6 and over as a high titer.

Trained nurses interviewed participants about their own and their family members' histories of liver disorders, prior blood transfusions including age at and volume of transfusion, acupuncture and alcohol intake.

Statistical Methods

Binary regression was used to examine effects of age or birth year, radiation dose, and health behaviors ascertained during interview. Prevalence of each HBV-related measurement with any particular values of radiation exposure or questionnaire variables was equal to the corresponding relative prevalence ratios times the baseline prevalence:

$$P(\text{city, sex, age or birth year, } e, d, t, u, h) = R_E(e) \times R_D(d) \times R_T(t) \times R_U(u) \times R_H(h) \times P_0(\text{city, sex, age or birth year}),$$

where the relative prevalences are log-linear functions of continuous radiation dose (e) or group indicators in the case of the factors drinking (d), transfusion (t), acupuncture (u), and family history (h). Each factor had a comparison group (baseline value) with relative prevalence 1; e.g., R_T (never transfused) = 1 for subjects who reported never having received a transfusion. Data were analyzed using the generalized logistic regression procedure Generalized Models for Binary Observations (GMBO) in the Epicure software (Hirosoft International, Seattle, WA) with an identity link (*13*).

Individual outcomes from prevalence data (presence or absence of disease) are binary and thus cannot be easily visualized graphically. Although the regression uses the individual binary data, it is necessary to group the data and calculate proportions when displaying them graphically. Baseline prevalence was computed for each group with adjustment for the other factors. Smooth estimates of trends were obtained using polynomials fitted to the original, ungrouped data. Likelihood methods were used to estimate 95% confidence intervals and P values (*13*).

We estimated the proportion of persons who were unable to clear the virus as the proportion of persons who were positive for HBsAg (HBV carriers) among persons who tested positive for HBsAg or core or surface HBV antibodies. To roughly separate persons ever infected with HBV into those infected with HBV through maternal transmission or other means before A-bomb radiation and those infected afterward, we divided this group according to their transfusion status. One group consisted of subjects who first received blood transfusions after irradiation in 1945 but before 1973 when screening of blood transfusions for HBV was begun in Japan (*14*). The other group consisted of persons who either said they did not receive transfusions or who received them before exposure to A-bomb radiation or after screening of transfusions for HBV. We assumed that such persons would be more likely to have been infected through maternal contact that occurred before exposure to A-bomb radiation. We excluded from this analysis persons first transfused in 1944 or in 1973–1974, because their status was unclear.

RESULTS

Table 1 shows the characteristics of the study population. About 80% of the AHS participants were more than 60 years old, and 65.5% were women. Thirteen percent received blood transfusions, 40% underwent acupuncture, and 36.5% were current alcohol drinkers.

Table 2 shows the adjusted prevalences of HBsAg, anti-HBsAb and anti-HBcAb by city and sex, adjusted for all statistically significant factors shown in the table. Overall, of the 6121 cohort members tested, 108 were positive for HBsAg, a crude prevalence of 1.8%. Table 2 also presents the relative prevalences (RPs), 95% confidence intervals, and associated P values of HBsAg, anti-HBsAb and anti-HBcAb by health behaviors and other factors associated with HBV infection and liver disease. RP estimates were adjusted for all statistically significant factors included in Table 2 and for birth year. Figures 1 and 2 show the relationships between birth cohort and the prevalence of HBsAg anti-HBsAb, and anti-HBcAb, with Figs. 3 and 4 similarly displaying the relationships between radiation exposure and these outcomes. As shown in Table 2, HBsAg positivity was significantly higher in Nagasaki and, as shown in Fig. 1, in younger birth cohorts (P = 0.002). Although there was not a statistically significant relationship between HBsAg prevalence and family history of liver disease, the adjusted prevalence of HBsAg was 1.58 times higher among persons who reported that their mother had liver disorders.

The prevalence of anti-HBsAb was approximately 1.10 times higher in Nagasaki (P = 0.022, Table 2). The prevalence was 35% for males and 31% for females, about 1.13 times higher in males (P = 0.002), decreasing slightly with

TABLE 1
Characteristics of the Study Population

	Number	Percentage
Age (years)		
<50	450	7.4
50–59	903	14.8
60–69	2661	43.5
70+	2107	34.4
Birth cohort		
–1915	863	14.1
1916–1925	1473	24.1
1926–1935	2509	41.0
1936–1945	1276	20.8
Sex		
Male	2112	34.5
Female	4009	65.5
City		
Hiroshima	3752	61.3
Nagasaki	2369	38.7
Dose (mGy)		
<0.005	2282	37.3
0.005–0.499	1412	23.1
0.5–0.999	685	11.2
1+	890	14.5
Unknown	852	13.9
Blood transfusion		
Never	5095	83.2
Ever	794	13.0
Unknown	232	3.8
Acupuncture		
Never	3590	58.7
Ever	2448	40.0
Unknown	83	1.4
Alcohol use		
Never	3581	58.5
Current	2234	36.5
Past	264	4.3
Unknown	42	0.7
Maternal history of liver disease		
No	5838	95.4
Yes	126	2.1
Unknown	157	2.6
Other family history of liver disease		
No	4969	81.2
Yes	995	16.3
Unknown	157	2.6

birth year ($P < 0.001$, Fig. 2). The prevalence of anti-HBsAb was significantly related to family history of liver disease and having had acupuncture (Table 2).

The prevalence of anti-HBcAb was 15% in Hiroshima and 14% in Nagasaki (Table 2). The prevalence was about 1.14 times higher in males ($P = 0.011$) and 1.27 times higher in persons with a family history of liver disease ($P = 0.002$). The prevalence of anti-HBcAb depended significantly on birth year, but in a curvilinear way ($P = 0.019$, Fig. 2). Similar results were found for anti-HBcAb with high titer.

After adjustment for birth year, city and sex, HBsAg prevalence increased 1.39-fold per gray ($P = 0.036$). After controlling for family history as well, anti-HBcAb increased 1.17-fold per gray (Table 3 and Figs. 3 and 4). The prevalence of a high titer of anti-HBcAb also increased significantly with radiation dose. In contrast, after further controlling for acupuncture, anti-HBsAb status was not significantly related to radiation dose (Fig. 4).

Table 3 shows by age, birth year, sex, city and radiation dose the numbers of subjects who at the time of blood drawing were either (1) currently infected with HBV [HBsAg-positive (+)], (2) previously infected with HBV [HBsAg negative (–) but antibody-positive for either anti-HBcAb or anti-HBsAb], or (3) never infected with HBV [HBsAg(–), anti-HBcAb(–), and anti-HBsAb(–)]. HBV carriers who were currently infected with HBV were more likely to be from Nagasaki ($P = 0.063$), and they had higher radiation exposures ($P = 0.064$) than subjects who were previously infected with HBV. Comparison of the two groups showed that younger subjects and those born in more recent years were significantly more likely ($P < 0.001$) to be currently infected with HBV.

Using blood transfusion as a reasonably approximate surrogate for HBV infection having occurred in adulthood, after radiation exposure, rather than during infancy, allows evaluation of the hypothesis that radiation exposure decreased the ability of immune surveillance against HBV infection to successfully clear a new HBV infection. Further analysis focused on persons who were ever infected, examining the proportion who were currently infected as a function of radiation dose. Among 1,848 subjects with evidence of ever having been infected with HBV whose transfusion histories were known, 105 had a transfusion between 1945 and 1972. The proportion of those who were unable to clear the virus increased with radiation dose in the subset who reported having had a transfusion ($P = 0.039$, $n = 105$), the dose response for the odds ratio was 2.37 at 1 Gy, but not clearing HBV was not significantly related to dose in the subset who reported no transfusions ($P = 0.16$, $n = 1{,}743$, P for difference in dose–response curves $= 0.043$) (Fig. 5).

DISCUSSION

The results of this study are consistent with those from two earlier studies of A-bomb survivors (*7, 8*) in showing that radiation exposure significantly increases the risk for HBV carriers. Our results suggest that radiation reduces the ability of HBV-infected persons to clear this virus, thereby increasing their likelihood of becoming chronically infected. Several previous studies of A-bomb survivors have shown significant associations between radiation exposure and increased mortality due to liver cancer (*1*) and cirrhosis

TABLE 2
Prevalence of HbsAg, Anti-HBsAb and anti-HBcAb among the AHS by City, Sex and Other Factors

	HBsAg	Anti-HBsAb	Anti-HBcAb
	Adjusted prevalence[a] (%)		
City			
Hiroshima	1.1%[b]	31%[b]	15%
Nagasaki	1.7%[b]	34%[b]	14%
	(*P = 0.043*)	(*P = 0.022*)	(*P = 0.37*)
Sex			
Male	1.6%	35%[b]	16%[b]
Female	1.1%	31%[b]	14%[b]
	(*P = 0.096*)	(*P = 0.002*)	(*P = 0.011*)
	Relative prevalence (95% CI)[c] *P* value		
History of liver disease (vs. none)			
Mother	1.58 (0.49–3.70)	1.04 (0.81–1.29)	1.04 (0.69–1.49)
	P = 0.40	*P = 0.73*	*P = 0.84*
Other family member	1.35 (0.82–2.14)	1.15 (1.05–1.26)[b]	1.27 (1.09–1.46)[b]
	P = 0.23	*P = 0.003*	*P = 0.002*
Transfusion			
(Received vs. none)	1.31 (0.71–2.22)	1.05 (0.94–1.16)	1.10 (0.92–1.30)
	P = 0.36	*P = 0.4*	*P = 0.28*
Acupuncture			
(Received vs. none)	0.84 (0.56–1.24)	1.08 (1.01–1.16)[b]	1.09 (0.97–1.22)
	P = 0.38	*P = 0.025*	*P = 0.14*
Alcohol consumption (vs. never drank)			
Current	1.09 (0.72–1.64)	1.03 (0.94–1.12)	0.93 (0.81–1.07)
	P = 0.67	*P = 0.54*	*P = 0.3*
Past	2.03 (0.85–4.10)	1.04 (0.86–1.23)	1.18 (0.89–1.53)
	P = 0.10	*P = 0.7*	*P = 0.24*
Radiation			
(relative increase per gray)	1.39 (1.02–1.81)[b]	1.05 (0.99–1.11)	1.17 (1.06–1.27)[b]
	P = 0.036	*P = 0.11*	*P = 0.002*

[a] Adjusted for all statistically significant factors.
[b] Statistically significant at $P < 0.05$.
[c] Relative prevalence estimates were adjusted for all statistically significant factors included in this table and for birth year.

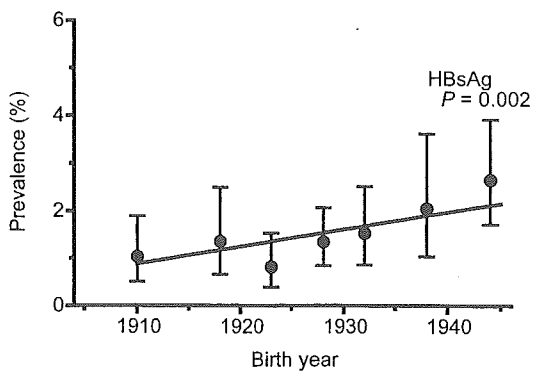

FIG. 1. Prevalence of HBsAg by birth year averaged over city and adjusted to a radiation dose of 0 Gy. Points display adjusted prevalence in summary birth year groups with bars representing 95% confidence intervals.

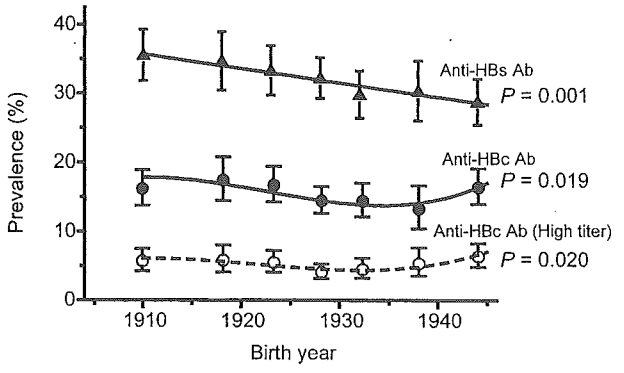

FIG. 2. Prevalences of anti-HBsAb, anti-HBcAb and high-titer anti-HBcAb by birth year. Prevalence estimates were averaged over city and gender and adjusted to a radiation dose of 0 Gy and no history of acupuncture. Points indicate adjusted prevalences in summary birth year groups with 95% confidence intervals.

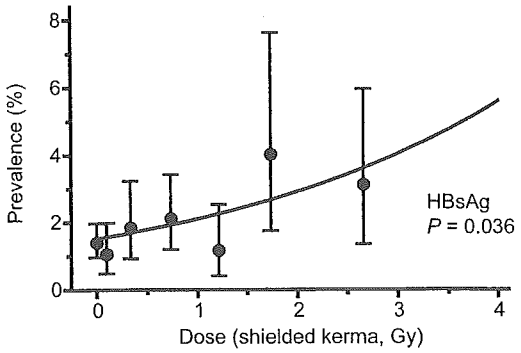

FIG. 3. Prevalence of HBsAg by radiation dose averaged over birth year and city. Points display adjusted prevalence in summary dose groups with 95% confidence intervals. The dose groups shown on the plot are: 0 (<0.005) Gy, 0.005–0.25 Gy, 0.251–0.75 Gy, 0.751–1.25 Gy, and 1.251–4.0 Gy, which were selected to provide approximately equal numbers of persons in each dose group. The points are plotted at the mean doses in these groups.

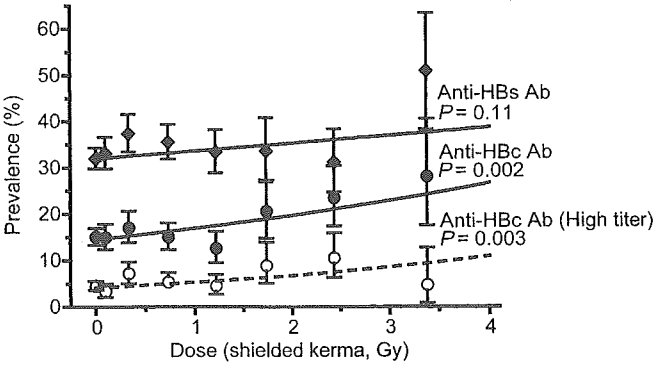

FIG. 4. Prevalence of anti-HBsAb, anti-HBcAb and high-titer anti-HBcAb by radiation dose, averaged over birth year and gender. Points indicate adjusted prevalence in summary dose groups with 95% confidence intervals.

(*15*) as well as increased incidence of liver cancer (*2, 3*) and chronic liver disease (*4*). However, because none of these studies controlled for HBV infections, a well-known cause of liver cancer, chronic hepatitis and cirrhosis, it is not clear if radiation is associated with these liver diseases only because of its association with HBV or independently.

Hepatocellular carcinoma develops frequently in patients with advanced stages of chronic liver diseases. In Japan, more than 60–75% of cases of HCC are related to chronic infection by HCV and 20–25% of cases are due to HBV

TABLE 3
Number of Subjects Currently, Previously or Never Infected with HBV by Age, Sex, City and Radiation Dose

	Ever infected								
	Current[a]		Past[b]			Never infected[d]		Total	
	Number	(%)	Number	(%)	*P* value[c]	Number	(%)	Number	(%)
Age (years)					<0.001				
<50	11	10.2	161	6.6		278	7.8	450	7.4
50–59	26	24.1	308	12.7		569	15.9	903	14.8
60–69	46	42.6	1031	42.4		1584	44.2	2661	43.5
70+	25	23.1	929	38.2		1153	32.2	2107	34.4
Birth cohort					0.001				
–1915	12	11.1	386	15.9		465	13.0	863	14.1
1916–1925	16	14.8	620	25.5		837	23.4	1473	24.1
1926–1935	45	41.7	976	40.2		1488	41.5	2409	41.0
1936–1945	35	32.4	447	18.4		794	22.2	1276	20.8
Sex					0.12				
Male	48	44.4	900	37.1		1164	32.5	2112	34.5
Female	60	55.6	1529	62.9		2420	67.5	4009	65.5
City					0.063				
Hiroshima	54	50.0	1435	59.1		2263	63.1	3752	61.3
Nagasaki	54	50.0	994	40.9		1321	36.9	2369	38.7
Dose (mGy)					0.064				
<0.005	34	31.5	863	35.5		1385	38.6	2282	37.3
0.005–0.499	20	18.5	563	23.2		829	23.1	1412	23.1
0.5–0.999	14	13.0	275	11.3		396	11.0	685	11.2
1+	21	19.4	348	14.3		521	14.5	890	14.5
Unknown	19	17.6	380	15.6		453	12.6	852	13.9
Total	108	100.0	2429	100.0		3584	100.0	6121	100.0

[a] Currently infected with HBV [HBsAg-positive (+)].
[b] Previously infected with HBV [HBsAg negative (−) but antibody-positive for either anti-HBcAb or anti-HBsAb].
[c] *P* values correspond to comparing the proportion between persons current infected and persons previously infected.
[d] Never infected with HBV [HBsAg(−), anti-HBcAb(−), and anti-HBsAb(−)].

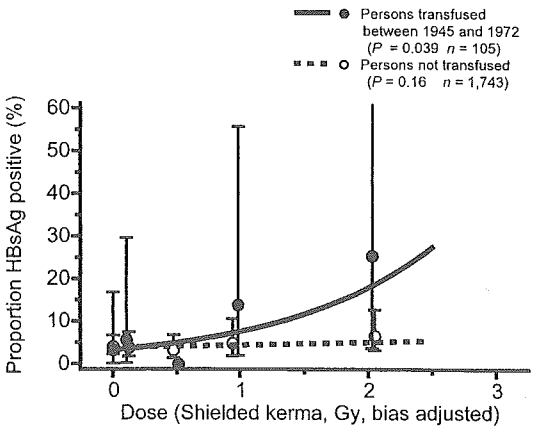

FIG. 5. Proportions of persons who were unable to clear HBV as a proportion of HBsAg+ among ever infected (HBsAg+ or anti-HBsAb+ or anti-HBcAb+). Comparison between persons transfused between 1945 and 1972 and all others (except those transfused in 1944 or 1973–1974). Points indicate prevalence in summary radiation dose groups with 95% confidence intervals. Proportions were adjusted for city and birth year.

infections (*16, 17*). Our previous report showed no relationship between atomic bomb radiation dose and prevalence of anti-HCV Ab using the same AHS participants as in the present study (*9*). Results of the present study show that although the prevalence of HBsAg increases with radiation dose, there is no association between radiation dose and the prevalence of anti-HBsAb, a finding that is consistent with results of previous studies. The present study is the first to show that the prevalence of anti-HBcAb increases with radiation dose. In general, a high titer of anti-HBcAb without corresponding positivity for anti-HBsAb indicates a continuation of HBV infection (*18*). The significantly increased prevalence of high-titer anti-HBcAb with increased radiation exposure that we report may reflect the higher prevalence of HBV carriers among persons exposed to higher doses of radiation.

Among HBV carriers, the primary route of HBV infection is transmission from mothers to their babies, after which 80–90% of them become chronically positive for HBsAg (*10*). Most people who are infected by HBV mainly through blood transfusion but also through routes of sexual or other transmission as adults are subsequently cured of acute HBV infection, with serum HBsAg being replaced by anti-HBsAb; about 10% of these become HBV carriers (*10*). In persons who have recovered from acute HBV infection, anti-HBsAb and/or anti-HBcAb persist indefinitely in serum. If the hypothesis of radiation-related suppression of immune function applies to HBV infection, then the proportion who are unable to clear the virus should increase with radiation dose. The present study demonstrated that, among those ever infected by HBV, the proportion of those chronically infected increased with radiation dose. We used information on blood transfusion as a rough surrogate measure to separate persons ever infected with HBV into those infected as infants through maternal contact before exposure to A-bomb radiation and those infected as adults afterward. Although using transfusion status in this way will no doubt misclassify some subjects in terms of the time their HBV infections occurred, this misclassification should be independent of radiation dose and thus would be expected to attenuate, not exaggerate, risk estimates. Our results suggest that among persons who were more likely to have been infected with HBV as adults by blood transfusion after irradiation, the proportion of those who were unable to clear the virus increased significantly with radiation dose. Our failure to find a similar dose–response relationship among those who were not transfused during this time frame, and thus who were more likely to have been infected as infants through their mothers, supports the idea that ionizing radiation interferes with the immune system's ability to clear HBV infections.

The immune response initiated by the T-cell response to viral antigens is thought to be fundamental for viral clearance and disease pathogenesis in HBV infection (*19*). The T-cell response during acute HBV infection is characterized by a vigorous, polyclonal and multispecific cytotoxic and helper T-cell response. By contrast, the immune response in HBV carriers, who are not able to eliminate the virus, is weak or undetectable. Thus a dominant cause of viral persistence could be the existence of a weak antiviral immune response. Although clearance of most virus infections is widely thought to indicate the killing of infected cells by virus-specific T cells, data suggest that non-cytolytic intracellular viral inactivation by cytokines released by virus-inactivated lymphomononuclear cells without killing the infected cell could have an important role in the clearance of this virus. In the RERF studies to date, a dose-dependent decrease in the function of T lymphocytes was observed among A-bomb survivors (*20, 21*). Also, a reactivation of the EB virus was observed among A-bomb survivors, which suggests that radiation compromises ability to defend against infection (*22*). By decreasing levels of immune competence, radiation exposure may have caused a dose-dependent increase in the prevalence of HBsAg among persons infected with HBV as adults. However, the question of whether the radiation-associated prevalence of HBsAg is due to suppression of immunological responsiveness requires further study including immunological examinations.

There are several limitations to the present study. About 50% of the original cohort of AHS subjects died before the time of blood collection in 1993–1995. Thus these results cannot be generalized to all atomic bomb survivors. However, the radiation dose–response relationship for HBsAg documented in the present study was similar to that obtained in previous reports conducted more than 30 years ago (*6, 7*). To distinguish between those who were infected by HBV from mothers as infants and those who were infected as adults, we used self-reported information about blood transfusion as a surrogate marker of adult infection. Although blood transfusion is a quite distinct and memorable event, and the recall for blood transfusion should be

relatively accurate, some of the persons receiving blood transfusions after 1945 were not infected with HBV as adults. There are also a number of other transmission routes including sexual transmission, and transmission by saliva, feces and other means than blood transfusion. Although uncertainty remains regarding the accuracy of self-reports and the bias of non-reporting cannot be ruled out, this study provides a basis for a better understanding the increased HBsAg prevalence among A-bomb survivors.

We examined the prevalence of HBsAg, anti-HBsAb and anti-HBcAb in this population-based study of atomic bomb survivors in Hiroshima and Nagasaki. After adjustment for sex and radiation dose, the prevalence of anti-HBsAb was higher in older birth cohorts in which a lower prevalence of HBsAg were found. Because of the design of our study, we cannot separate age at the time of HBV measurement from birth cohort effects. However, because chronic HBV infection occurs primarily before 3 years of age and HBV prevalence varied over time, year of birth is probably more important than age in explaining differences in HBV prevalence. A cubic term of the baseline prevalence of anti-HBcAb with birth year was statistically significant. Presumably this shape has something to do with secular changes in infection rates and routes of infection, but in a cross-sectional study it is difficult to infer such effects.

In conclusion, the proportion of persons who were unable to clear HBV increased with radiation dose among those receiving blood transfusions, which may suggest a reduced ability to clear HBV infections for the people most likely to have been infected with HBV as adults after irradiation. Further studies will be needed, however, to answer the question of whether radiation affects a person's ability to clear HBV upon infection through suppression of immunological responsiveness to HBV infection among the atomic bomb survivors.

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HEPATOCELLULAR CARCINOMA AMONG ATOMIC BOMB SURVIVORS: SIGNIFICANT INTERACTION OF RADIATION WITH HEPATITIS C VIRUS INFECTIONS

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We conducted a nested case-control study within the cohort of Japanese survivors of the 1945 atomic bombings to study the joint effects of HBV and HCV with radiation on the risk of HCC. Among subjects who received autopsies during 1954–1988, we analyzed archival tissue samples for 238 pathologically confirmed HCC cases and 894 controls who died from diseases other than liver cancer. Using logistic regression and adjusting for potential confounders and other factors, we found a statistically significant, supermultiplicative interaction between A bomb radiation and HCV in the etiology of HCC. Compared to subjects who were negative for HCV and radiation, ORs of HCC for HCV-positive subjects showed a statistically significant, greater than multiplicative increase for liver irradiation exposures in the second (>0.018–0.186 Sv, $p = 0.04$) and third (>0.186 Sv, $p = 0.05$) tertiles of non-zero radiation exposure but not for first tertile exposure (>0–0.018 Sv, $p = 0.86$). Limiting analysis to subjects without cirrhosis, HCV-infected subjects were at 58.0-fold (95% CI 1.99–∞) increased risk of HCC per Sv of radiation exposure ($p = 0.017$), a supermultiplicative interaction between radiation and HCV that was not found among subjects with cirrhosis ($p = 0.67$). We found no evidence of interaction between HBV infection and radiation exposure in the etiology of HCC, regardless of cirrhosis status ($p = 0.58$). We conclude that among survivors of the nuclear bombings of Hiroshima and Nagasaki, subjects who were both HCV-positive and radiation-exposed were at a significantly, supermultiplicatively increased risk of HCC without concurrent cirrhosis.

Key words: *radiation; hepatitis B; hepatitis C; hepatocellular carcinoma*

Although HBV and HBC are the most important causes of HCC worldwide, each elevating risk of this cancer >10-fold,[1] no previous study of ionizing radiation and liver cancer has taken either virus into account. We address the question of whether HBV and HCV infections might affect the relationship between acute exposure to radiation and HCC. This is important to improve our understanding of the mechanisms of hepatocarcinogenesis underlying one of the world's most common and most deadly cancers. It is also important in addressing discrepancies in the results of studies of acute radiation exposure and liver cancer, where some cohort studies conducted in areas of high HBV or HCV prevalence show significant risk elevations of liver cancer while others conducted in low-prevalence areas do not.

Studies of liver cancer risks in atomic bomb survivors have consistently shown that exposure to low-LET ionizing radiation significantly increases liver cancer mortality rates[2] and primary liver cancer incidence, 85% of which is HCC.[3,4] In contrast, Western studies of radiotherapy patients have just as consistently shown liver cancer risks to not be elevated after acute radiation exposure to substantially higher doses during medical treatment.[5–8]

Previous studies have found synergy between hepatitis viruses and other risk factors, including *(i)* a supermultiplicative (or greater than multiplicative) relationship between HBV and aflatoxin exposure,[9–12] *(ii)* a moderate interaction between HCV and smoking[13,14] and *(iii)* a superadditive and submultiplicative relationship of both HBV and HCV with alcohol.[15] A meta-analysis of 32 studies concluded that the joint effects of HBV and HCV in the etiology of HCC is between additive and multiplicative.[1]

Because HBV and HCV infection rates are higher in Japan than in the Western countries, where studies have shown no relationship between acute radiation exposure and liver cancer, and because the liver appears to be especially prone to the interactive effects of multiple risk factors, our goal was to determine if radiation effects on HCC were increased by these infections.

MATERIAL AND METHODS

Selection of cases and controls

The RERF LSS cohort is a group of 120,321 atomic bomb–exposed and unexposed persons who were official residents of Hiroshima or Nagasaki in 1945 and who were alive and residing there at the time of censuses conducted in 1950–1952.[2,16]

From a collection of archival tissue samples and clinical records for 7,647 LSS cohort members autopsied in 1954–1988 in Hiroshima and Nagasaki, we assembled material for subjects whose

Abbreviations: CI, confidence interval; DS86, dosimetry system 1986; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; LET, linear energy transfer; LSS, Life Span Study; OR, odds ratio; RERF, Radiation Effects Research Foundation; RR, relative risk; Sv, sievert.

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cause of death was either liver cancer or one of several diseases frequently confused with liver cancer, including cirrhosis, chronic hepatitis, pancreatic cancer and gallbladder or biliary disease. This material was reviewed by a panel of 3 pathologists, as described in more detail elsewhere.[17,18] In brief, each pathologist independently reviewed medical material for each subject, and the panel then discussed each subject and reached consensus opinions on type of liver cancer, if present, cirrhosis status and HBV status (based on results of Orcein and immunohistochemical staining). The panel accepted 238 autopsied cases as primary HCC. Potential, nonautopsied cases of liver cancer were also identified through tumor registries; and tissue samples, when available, were stained and reviewed. For controls, we chose 894 persons without liver cancer from the group of 7,647 who were determined not to have liver cancer. Prior to case review, controls were matched to all potential cases on sex and city of residence at the time of the bombings and further selected to achieve balance with the potential cases on radiation exposure, age at death and, to the extent possible given the restricted period of the autopsy program, year of death. As described in more detail elsewhere,[19] a weighted distance score between all potential cases and controls was computed, and controls were selected who were closest to the cases on this distance score, individually within sex and city strata. Originally, slightly more than twice as many controls as potential cases were selected, to end up with about a 2:1 ratio after allowing for the possibility of unusable tissue. However, because there was no suitable source of comparable controls with nonautopsy liver tissue, we excluded all nonautopsied cases from the present analysis. We also excluded autopsied liver cancer cases who were determined by the pathology review panel to have cholangiocarcinoma and other subtypes of liver cancer other than HCC. In summary, our study was limited to 238 autopsied, pathologist-confirmed HCC cases and 894 autopsied, pathologist-reviewed controls, where controls were sampled according to sex, city, radiation dose, age at death and year of death but not necessarily matched to cases.

Histologic classification was made in accordance with the standards proposed by the WHO.[20] Histologic evidence of cirrhotic changes was obtained from nonneoplastic liver tissue, and the changes were characterized according to the 4 types proposed by Anthony *et al.*[21]

Determination of HBV and HCV status

To detect HBsAg, the pathology panel reviewed tissue slides stained with Orcein[22] and slides stained by anti-HBV immunohistochemical material (LSAB kit, Universal K681; Dako, Carpinteria, CA). Slides were prepared from nonneoplastic, formalin-fixed and paraffin-embedded liver tissues. To increase the accuracy of HBV testing, we also used PCR to test archival tissues from nontumor areas of the liver for the presence of genes encoding HBV antigens, as described elsewhere.[23] DNA was extracted from 5-μm-thick sections of tissue, and 3 separate HBV loci were amplified, the S, pre-C and X regions, using the following specific primer pairs for each locus: S region, MD03, 5′-CTTGGATCCTATGGGAGTGG-3′ and MD06, 5′-CTCAAGCTTCATCATCCATATA-3′; pre-C region, P20, 5′-AGGCATAAATTGGTCTGCGC-3′ and M1, 5′-ACGAGAGTAACTCCACAGTAGCTCC-3′; and X region, XP22, 5′- CCAGCAATGTCAACGACCG-3′ and XM0, 5′-ATTTATGCCTACAGCCTCC-3′. If any of these 3 regions of the HBV genome could be amplified or if either the Orsein or immunohistochemical stain was positive for HBV, the subject was considered positive for HBsAg. According to a WHO consensus opinion, the presence of HBsAg indicates active HBV infection.[24]

Agreement between the Orcein and immunohistochemical staining methods for HBV was good (κ statistic = 0.87, n = 1,014). Assuming that persons positive on at least one of the staining tests were HBV-positive, agreement between PCR tests for HBV and staining tests was also good (κ = 0.95, n = 1,017). Using radioimmunoassay procedures and commercially available reagents, we performed HBsAg tests on 51 subjects for whom frozen (−80°C) or freeze-dried serum samples were available. All 45 persons whose serum was negative for HBsAg were negative by tissue-based tests; 5 (83%) of the 6 persons whose serum was HBsAg-positive were positive by tissue-based staining or PCR. Because tissue staining and PCR were more likely to falsely classify HBV-positive subjects as negative instead of falsely classifying negative subjects as positive, we classified anyone testing HBV-positive by staining or PCR as positive to maximize the sensitivity of our tests. We classified everyone else testing negative as negative since the specificity of our tests was high.

To determine HCV status, we extracted RNA from a single 5-μm-thick section of paraffin-embedded liver tissue.[25] The methods of detecting the HCV genome and ensuring the integrity of mRNA in each sample and primer set have been described in detail elsewhere.[26] Briefly, the 5′-untranslated region of the HCV genome was amplified using our specific primer sets. After RT-PCR amplification of HCV, positive samples were identified by hybridizing with a radiolabeled oligomer probe that recognizes a sequence between the 2 primers. RNA integrity was assessed by amplification of *c-BCR* mRNA between 2 sequential exons with an intervening intron by RT-PCR.

We obtained and tested frozen serum samples (−80°C) from 43 subjects for whom HCV results of tissue-based RT-PCR tests were available. These samples were tested, under code, for HCV antibodies by ELISA-2 using commercially available reagents and by qualitative RT-PCR. Fifteen (65%) of the 23 subjects testing HCV-negative by both serum tests were negative by RT-PCR; the others tested positive by tissue-based RT-PCR. Of the 20 subjects testing positive by either serum test, 14 (70%) also tested positive by tissue-based RT-PCR, the other 6 being negative. Because serum samples were available for just a small fraction of subjects for whom tissue samples were available, analysis of the case-control study was based entirely on RT-PCR of archival samples of liver tissue.

Radiation exposure

Measures of liver irradiation from the atomic bombs were derived from the DS86.[27] This dosimetry system provides estimates of liver dose based on physical calculations of neutron particle and γ-ray bomb yields, interviews with cohort members about their locations and shielding by buildings and terrain during the bombings and estimates of radiation shielding by body tissue. We allowed for the differential effectiveness of γ-rays and neutron particles using a relative biologic effectiveness weighing factor of 10, multiplying the neutron dose by this number and adding it to the γ dose, as described in more detail elsewhere.[4] Among controls, the mean γ and neutron liver doses were 0.104 Gy (SD = 0.275) and 0.0007 Gy (SD = 0.003), respectively; among cases, these values were 0.123 Gy (SD = 0.335) and 0.0011 Gy (SD = 0.004).

Statistical methods

We used unconditional logistic regression to calculate ORs and 95% CIs of HCC for the risk factors under investigation, adjusting simultaneously for HBV and HCV and for the potential confounders on which controls were selected. Adjustment was made by adding main effects terms to the logistic regression model. Because viral hepatitis has a different relationship with HCC depending on whether cirrhosis is present or not,[28] we included cirrhosis status in statistical models or calculated ORs separately for subjects with and without cirrhosis. Because controls were selected to have a distribution of radiation exposure similar to that of cases, we either calculated ORs of HCC for exposure groups after separating subjects based on 4 exposure strata (see below) or estimated a trend with radiation dose using the mean radiation doses from the 4 strata in logistic models. Under DS86, kerma doses up to 0.005 Gy (<0.003 Sv liver dose) were recorded as 0; thus, some subjects in our 0-dose radiation group may have received inconsequential liver doses of 0.001–0.002 Sv.

To examine the joint effects of A bomb radiation and viral hepatitis, we constructed 4 exposure groups: unexposed (0 dose) and 3 groups of exposed (non-0 dose) subjects based on tertiles of radiation exposure calculated for exposed controls. Tertiles among the exposed (non-0 dose) were >0–0.018 Sv, >0.018–0.186 Sv and >0.186 Sv (Table I). We created radiation–virus interaction terms, which were the product of the mean dose in each exposure stratum and a 0 or 1 variable, representing exposure to HBV or HCV. We used unconditional logistic regression to calculate ORs and 95% CIs for these interaction terms, taking into account HBV and HCV, cirrhosis status and the control sampling factors of radiation dose, year of death, age at death, city and sex were also calculated p values for each interaction term included in logistic models to determine whether or not we could reject the null hypothesis that interaction was multiplicative in favor of the alternative hypothesis of supermultiplicative interaction, setting 0.05 as the level of statistical significance.

To calculate ORs for combined HCV and tertile-specific radiation exposures, we added the HCV main effect parameter to each HCV–mean dose interaction parameter and calculated the exponent of the result. The 95% CIs for these ORs were calculated using the formula for the SE of a sum of 2 variables: square root [variance (b_{HCV} + $b_{interaction}$) = variance (b_{HCV}) + variance ($b_{interaction}$) + 2 × covariance (b_{HCV}, $b_{interaction}$)]. The CI was the exponent of [the sum of the 2 parameters ± 1.96 times SE]. The expected values under the multiplicative model for these ORs could not be directly estimated because of control selection on radiation dose. To estimate the main effect for radiation, we used the latest excess RR estimates of liver cancer for A bomb radiation,[3] adjusted to the mean doses in the tertiles. These OR estimates were multiplied by the OR for HCV.

We calculated profile likelihood 95% CIs and likelihood ratio p values. Model fit was evaluated using the Hosmer and Lemeshow goodness-of-fit test. SAS version 6.12 was used to perform all analyses (SAS Institute, Cary, NC).

RESULTS

Table I shows the distribution of sex, city, liver irradiation, age at death, year of death and HBV/HCV status among cases and controls. Among cases, 75.5% had cirrhosis compared to 7.1% of controls.

HCV and radiation

We found the integrity of RNA, as measured by the percentage of *c-BCR* mRNA amplifiable by RT-PCR of liver samples, to be 59.5%. We determined HCV status for 61.7% of controls and 62.6% of cases.

Table II presents a comparison of 2 logistic models examining the joint effects of HCV and liver irradiation in the etiology of HCC. The full model included cirrhosis status, the control selection factors, HBV/HCV status and the HCV–radiation interaction term; the reduced model excluded the nonsignificant factors radiation dose, age at death and sex. As shown in Table II, both the full and reduced models showed borderline statistically significant results for the HCV–radiation interaction term. Under the reduced model, the OR of HCC among the HCV-infected increased 5.7-fold per Sv increase in radiation exposure (95% CI 0.86–37.91). The corresponding increase under the full model was 10.0 per Sv increase, but the 95% CI for this OR was much wider with the inclusion of the additional factors.

As shown in Table III, when analysis was limited to the 528 subjects without cirrhosis, there was a statistically significant, positive interaction between liver irradiation and HCV (p = 0.017). Thus, we can reject the null hypothesis that the joint effect is multiplicative and accept the alternative hypothesis that it is

TABLE I – CONTROL SELECTION AND POTENTIAL RISK FACTORS FOR AUTOPSIED SUBJECTS DYING FROM HCC (CASES) OR FROM DISEASES OTHER THAN LIVER CANCER (CONTROLS), HIROSHIMA AND NAGASAKI, JAPAN, 1954–1988

Characteristic	Controls		Cases	
	Number	%	Number	%
Sex				
Female	261	29.2	69	29.0
Male	633	70.8	169	71.0
Age at death (years)				
20–39	31	3.5	4	1.7
40–59	235	26.3	79	33.2
60–90	628	70.2	155	65.1
Decade of death				
1950s	26	2.9	3	1.3
1960s	350	39.2	60	25.2
1970s	453	50.7	82	34.5
1980s	65	7.3	93	39.1
City of residence at time of bombing				
Hiroshima	576	64.4	163	68.5
Nagasaki	318	35.6	75	31.5
Liver irradiation level (mean Sv)[1]				
Unknown	52	5.8	13	5.5
0 (0)	465	52.0	127	53.4
Tertile 1 (0.009)	124	13.9	27	11.3
Tertile 2 (0.071)	127	14.2	35	14.7
Tertile 3 (0.686)	126	14.1	36	15.1
HBV				
Negative	730	81.7	148	62.2
Positive	42	4.7	62	26.0
Unknown	122	13.6	28	11.8
HCV				
Negative	510	57.0	82	34.4
Positive	42	4.7	67	28.3
Unknown	342	38.3	89	37.4
Total	894		238	

[1]Under the current dosimetry system (DS86), liver doses up to 0.003 Sv are recorded as 0. Radiation exposures within tertiles were 1, >0–0.018 Sv; 2, >0.018–0.186 Sv; and 3, >0.186 Sv.

TABLE II – JOINT EFFECTS OF ATOMIC BOMB RADIATION AND HCV INFECTIONS ON RISKS OF HCC, AUTOPSIED CASES AND CONTROLS, 1954–1988

Risk factor	Full model[1] (n = 670)		Reduced model[2] (n = 693)	
	OR (95% CI)	p	OR (95% CI)	p
HCV infection (yes/no)	5.9 (2.68–13.39)	0.0001	6.2 (2.80–13.93)	0.0001
HBV infection (yes/no)	5.8 (2.68–12.67)	0.0001	5.5 (2.60–12.00)	0.0001
HCV–radiation interaction term (per Sv with HCV infection)[3]	10.0 (0.87–137.76)	0.072	5.7 (0.86–37.91)	0.065
Cirrhosis	45.2 (23.66–91.86)	0.0001	44.8 (23.78–89.35)	0.0001

[1]Also adjusted for the study's control selection factors: radiation exposure (p = 0.50), year of death (p = 0.0001), age at death (p = 0.78), city (p = 0.0001) and sex (p = 0.40).–[2]Additionally adjusted only for statistically significant factors: year of death and city.–[3]Mean liver irradiation level for exposure category (as shown in Table I) multiplied by a binary (0, 1) term representing HCV infection status.

TABLE III – JOINT EFFECTS OF ATOMIC BOMB RADIATION AND HCV INFECTIONS ON RISKS OF HCC ACCORDING TO PRESENCE OF CIRRHOSIS, AUTOPSIED CASES AND CONTROLS, 1954–1988

Risk factor	Cirrhosis not present (n = 528)[1]		Cirrhosis present (n = 142)[1]	
	OR (95% CI)[2]	p[2]	OR (95% CI)[2]	p[2]
HCV infection (yes/no)	3.5 (0.98–10.96)	0.054	9.0 (2.86–35.77)	<0.0001
HBV infection (yes/no)	14.2 (4.93–40.90)	<0.0001	3.0 (1.07–9.66)	0.036
HCV–radiation interaction term (per Sv with HCV infection)[3]	58.0 (1.99–∞)	0.017	0.4 (0.0007–59.26)	0.67

[1]Numbers of subjects for whom information available for all factors included in logistic models: no cirrhosis, 30 cases and 498 controls; cirrhosis-positive, 108 cases and 34 controls.–[2]Also adjusted for the study's control selection factors: radiation exposure, year of death, age at death, city and sex.–[3]Mean liver irradiation level for exposure category (as shown in Table I) multiplied by a binary (0, 1) term representing HCV infection status.

TABLE IV – JOINT EFFECTS OF ATOMIC BOMB RADIATION AND HCV INFECTIONS ON RISKS OF HCC, AUTOPSIED CASES AND CONTROLS, 1954–1988[1]

HCV/radiation status	Controls		Cases		Parameter estimate (95% CI)	p	OR[2] (95% CI)
	Number	%	Number	%			
HCV^- and no radiation	239	85.6	41	39.8			1.0
$HCV^{+/-}$ and 1 Sv liver irradiation[3]	—	—	—	—		—	1.8 (1.32–2.43)
HCV^+ and no liver irradiation	25	9.0	34	33.0	1.55 (0.689–2.438)	0.0005	4.7 (1.99–11.44)
HCV^+ and tertile 1 liver irradiation[4]	4	1.4	6	5.8	1.55 + 0.19 (−1.847–2.344)	0.86	5.7 (0.76–43.0)
HCV^+ and tertile 2 liver irradiation[4]	2	0.7	10	9.7	1.55 + 2.46 (0.127–4.915)	0.04	55.1 (5.9–523.1)
HCV^+ and tertile 3 liver irradiation[4]	9	3.2	12	11.6	1.55 + 1.81 (0.058–3.677)	0.05	28.7 (5.8–141.2)
Total	279	100%	103	100%			

[1]The logistic model included the following factors HCV and HBV infection status, the 3 interaction terms, cirrhosis status and the study's control selection factors: radiation exposure, year of death, age at death, city and sex.–[2]Corresponding ORs, 95% CIs and parameter p values among subjects negative for cirrhosis were 2.9 (0.66–10.8; p = 0.12), (no radiation, HCV^+), undefined (p = 0.99, tertile 1 radiation, HCV^+), 41.3 (3.9–436.8; p = 0.05, tertile 2 radiation, HCV^+) and 61.2 (6.5–580.1; p = 0.02, tertile 3 radiation, HCV^+).–[3]Because radiation dose was a factor for control selection, the RR of HCC for radiation exposure could not be estimated. The RR of liver cancer of 1.8 per Sv is taken from the latest cohort analysis of liver cancer among A bomb survivors,[3] an analysis that did not adjust for HCV (about 9% of the cohort was HCV-infected).[33]–[4]Means and ranges of liver irradiation exposures for tertiles are listed in Table I.

greater than multiplicative. HCV-infected, noncirrhotic persons were at 58-fold increased risk of HCC per Sv of liver irradiation, though the 95% CI for this OR was wide (1.99–∞). There was no significant interaction between HCV and radiation in the etiology of HCC accompanied by cirrhosis (OR = 0.4, p = 0.67). These logistic models controlled for all 5 control selection factors, including liver irradiation, as well as for HCV and HBV. Although HCV infections were significantly associated with both noncirrhotic and cirrhotic HCC, the virus was a stronger risk factor for HCC among subjects with cirrhosis (ORs = 3.5 and 9.0, respectively).

As shown in Table IV, compared to subjects who were negative for both HCV and liver irradiation, HCV-positive subjects with no liver irradiation were at 4.7-fold greater risk of HCC (p = 0.0005) after adjusting for cirrhosis. ORs and 95% CIs of HCC for HCV-positive subjects with tertiles 1, 2 and 3 radiation exposure were 5.7 (0.76–43.0), 55.1 (5.9–523.1) and 28.7 (5.8–141.2), respectively. Based on the excess RR of 0.8 per Sv liver irradiation found in the most recent cohort analysis of liver cancer risk among A bomb survivors[3] and under the multiplicative model, we would expect an RR of 7.3 [4.7 × (1 + 0.8 × 0.686)] for the joint effects of HCV and the mean dose of liver irradiation in the highest exposure group (tertile 3). When we restricted analysis to subjects without cirrhosis and controlled for the factors listed in Table IV, the corresponding ORs and 95% CIs were 2.9 (0.66–10.8; no radiation, HCV^+), undefined (p = 0.99; tertile 1 irradiation, HCV^+), 41.3 (3.9–436.8; tertile 2 irradiation, HCV^+) and 61.2 (6.5–580.1; tertile 3 irradiation, HCV^+). Among subjects without cirrhosis under the multiplicative model, we would expect an RR of 3.6 [2.9 × (1 + 0.8 × 0.686)] for the joint effects of HCV and the mean liver irradiation level in tertile 3.

HBV and radiation

HBV status was determined for 86.4% of controls and 88.2% of cases. We found no evidence of interaction between HBV and liver irradiation in the etiology of HCC. The p value for the HBV–radiation term was 0.58, adjusting for HCV, HBV, cirrhosis status and the 5 control selection factors, including liver irradiation. This p value was 0.30 when cirrhosis status was excluded from the model. In contrast to HCV, HBV infection was a stronger risk factor for HCC among those without cirrhosis (ORs 14.2 and 3.0, respectively) (Table III).

DISCUSSION

We found that HCV infection combined with liver irradiation significantly elevated HCC risks after controlling for the effects of HCV and liver irradiation alone, as well as for cirrhosis status and other factors. HCC risks were 28.7-fold higher ($p = 0.05$) among HCV-infected persons exposed to the highest one-third of non-0 liver radiation doses compared to those negative for liver irradiation and HCV. In contrast, under a conservative multiplicative model, which uses the possibly inflated RR at 1 Sv of 1.8 reported by a cohort study of liver cancer in HCV-positive and -negative A bomb survivors,[3] the expected RR for this comparison would be 7.3. HCC risks were also significantly elevated in HCV-infected subjects exposed to the middle-third of non-0 liver radiation doses ($p = 0.04$); HCC risks were not significantly elevated for HCV-infected subjects with lower radiation exposures ($p = 0.86$). Among subjects without cirrhosis, HCV-infected subjects with tertile 2 and 3 radiation exposures were at 41.3-fold ($p = 0.05$) and 61.2-fold ($p = 0.02$) greater risk of HCC, respectively, again in comparison to HCV-negative and radiation-unexposed subjects and after subtracting out the effects of liver irradiation and HBV and HCV infection alone. Under a multiplicative model, the expected OR for tertile 3 radiation exposure and HCV infection would be 3.6. Thus, our results are consistent in indicating a greater than multiplicative relationship between HCV and liver irradiation in the etiology of HCC, which is especially pronounced among subjects without cirrhosis.

In contrast, we found no increased risks of HCC for liver irradiation among HBV-positive persons after factoring out the effects of HBV and liver irradiation alone on hepatocarcinogenesis.

The major difficulty in conducting this research was the limited number of cases and controls for whom radiation exposures were known and liver tissue samples were available to allow pathology review and assessment of cirrhosis and viral hepatitis. To increase statistical power, we selected 2 controls per potential case. After pathology review and restriction of cases to subjects with pathology-confirmed HCC who, like controls, had received autopsies, the study included nearly 4 times as many controls as cases. To further increase statistical power, we selected controls to have a distribution of radiation exposures similar to that of cases, to increase the number of controls exposed to higher levels of liver irradiation, a number likely to be too low had they been selected randomly. Matching the distributions of radiation dose for cases and controls has been demonstrated to be effective at increasing power to detect statistical interaction.[29] Nevertheless, our data are fairly sparse, as can be seen by the wide CIs accompanying many of the ORs. Our finding of no interaction in the etiology of HCC accompanied by cirrhosis should be interpreted cautiously since this analysis was based on just 142 subjects with both cirrhosis and complete exposure information. In comparison, our risk estimates for subjects without cirrhosis were based on exposure assessments of 528 subjects and have greater statistical power. Although we consistently found a statistically significant, greater than multiplicative interaction between liver irradiation and HCV in the etiology of HCC, the degree to which risks of this cancer are increased by these joint exposures must be viewed as poorly quantified by this study due to sparse data.

A second problem of our study was the unequal distribution of cases and controls by year of death because the autopsy program was more active in earlier years of cohort follow-up and the incidence of HCC increased in the cohort over time. To address this problem, we insured that the range of years of death was identical for both cases and controls (1954–1988), and we included year of death in all analyses. According to Yoshizawa,[30] HCV was widespread in Japan in the 1950s due to use of illegal drugs after World War II. We found that 9.2% of subjects dying in the 1960s were HCV-infected, which also suggests that HCV was present in Japan this early. Since 868 controls and 235 cases died after 1959, a large number of subjects would have been alive when HCV was present in this population. Our findings suggest that risks of HCV-induced HCC increase with radiation dose. Since we controlled for both radiation dose and year of death in all analyses and there was not a significant association between year of death and radiation dose, it appears unlikely that the difference in years of death of cases and controls could account for our results.

Tissue-based measures of HBV showed better agreement with serum-based measures in our limited validation than did tissue-based measures of HCV. Radiation exposure measures were based on interviews conducted and measurements made in the early 1950s at the beginning of cohort follow-up. Cirrhosis and HCC disease classifications were based on review by 3 pathologists of the ample liver tissue samples obtained during autopsies, and the pathologists were required to reach consensus opinions. Our results might be affected if the somewhat older tissue samples for controls were more likely to falsely test negative for HCV than the tissues of cases. However, RNA integrity was assessed by amplification of *c-BDR* mRNA; and when this RNA, which is present in all living cells, could not be amplified, samples were discarded. Success rates for HCV testing were similar for cases and controls (62.6% and 61.7%, respectively). Thus, the misclassification of HCV, as well as of the other risk factors and of cirrhosis status, that did occur is likely to have occurred randomly among cases and controls, thereby biasing OR estimates toward a finding of no association.

The time of radiation exposure of the A bomb survivor cohort is known precisely. Although HCV infections can become chronic regardless of the age at which they occur and therefore could have occurred at any time, there are several reasons to suspect that many infections followed A bomb irradiation in 1945: *(i)* mean age at bombing of HCV-infected cases was 33.8 years, and mean age at death was 66.1 years (thus, on average, about half their lifetimes were lived after the bombings); *(ii)* trauma from the explosions was associated with blood transfusions that were not screened for HCV; *(iii)* percentages of HCV-positive subjects increased with decade of death (from 9.2% in the 1960s to 14.6% in the 1970s to 30.4% in the 1980s). Therefore, it appears reasonable, albeit speculative, to suggest that in many instances hepatocytes were mutated by A bomb radiation but the process of carcinogenesis did not continue until subjects were infected with HCV and the virus started its cycle of hepatocyte destruction and regeneration. If cell mutation or epigenetic change occurred before the cellular proliferation associated with HCV infection rather than after it, the process of carcinogenesis might progress to HCC without going through the stage of cirrhosis. Thus, our failure to find interaction between radiation and HCV in the etiology of HCC accompanied by cirrhosis may not mean that no such interaction occurs. The explanation may lie in a time sequence of radiation exposure followed by HCV infection that is specific to the A bomb survivor cohort.

Our findings that the effect of acute radiation exposure on HCC risk was significantly increased by HCV infection may explain the consistently negative findings from large-scale mortality and incidence studies of acute radiation effects on liver cancer conducted in areas of low HCV prevalence. The findings from those studies are in conflict with the consistently elevated liver cancer risks found among the Hiroshima and Nagasaki survivors who were also acutely exposed to ionizing radiation but generally at much lower mean levels.[2–4] No excess liver cancer risk was found in U.S., U.K. or European populations exposed to high levels of acute liver irradiation during treatment of ankylosing spondylitis (mean liver dose 2.1 Gy),[6] peptic ulcer (mean liver dose 4.6 Gy),[5] benign gynecologic bleeding disorders (mean liver dose 0.21 Gy)[31] and cervical cancer (mean liver dose 1.5 Gy).[7] In comparison, mean liver doses in our study were 0.14 Sv for cases and 0.11 Sv for controls. (Although doses in the radiotherapy studies were in Gy, these would be equal to adjusted equivalent doses in Sv because X radiation has identical properties to γ radiation of the same energy, and both have relative biologic effectiveness weighing factor of 1.)

According to the review of Wasley and Alter,[32] HCV prevalence in the United States is about 1.8%, with lower prevalences reported for Western Europe (0.2–0.5%) and the lowest HCV prevalences found in the United Kingdom and Scandinavia (0.01–0.1%). In contrast, the prevalence of HCV in the A bomb survivor cohort is 4–5 times higher than in the United States and 80 or more times higher than in the United Kingdom, ranging from 7.8% for the controls in our study to 8.9% in an earlier clinical study of 6,121 A bomb survivors.[33]

Our findings suggest that excess RRs of primary liver cancer for radiation among HCV-negative A bomb survivors are lower than the previous mortality study excess risk estimates of 0.27 per Gy[2] and the incidence study excess risk estimates of 0.66 per Sv[4] and 0.81 per Sv[3] because these studies did not take into account HCV status and, conversely, radiation risks would be higher than this among HCV-positive persons. We did not find significant elevations in HCC risk for HCV-infected subjects in the lowest tertile of non-0 A bomb liver irradiation exposure (≤0.018 Sv), but risks were significantly elevated for HCV-positive subjects with mid-tertile and top-tertile radiation exposures. Supermultiplicative interaction was found both when we included cirrhotic and noncirrhotic subjects together in the analysis and adjusted for cirrhosis and when we limited analysis to noncirrhotic subjects.

Roles of viral hepatitis and other risk factors in hepatocarcinogenesis

Generally in epidemiologic studies of cancer the strongest interactions between risk factors are found when 2 agents play active roles at different steps in the carcinogenic process. In HCC, synergistic or supermultiplicative interactions between risk factors have been reported when 1 agent is primarily associated with genetic alteration of hepatocytes and the other with cellular proliferation and liver regeneration leading to clonal expansion.[12,34,35] Ionizing radiation has long been known for its ability to cause mutations and malignant transformation of cells.[36]

HBV has been called a "complete carcinogen" and appears to be involved in multiple steps in oncogenic progression to HCC.[37] Persistent HBV infection causes inflammation, increased cell turnover and cirrhosis. In addition, the HBV genome may be incorporated into the chromosomes of hepatocytes and may then cause genomic instability as a result of point mutations, deletions, translocations and rearrangements at multiple sites.[38] In contrast, the role of HCV in HCC appears to primarily relate to its ability to cause inflammation, cellular injury and cirrhosis,[38] leading to cellular proliferation and hepatocyte regeneration, a function that higher cirrhosis rates for HCV indicates is more associated with HCV than with HBV.[39] Unlike both HBV infection and radiation, HCV, an RNA virus, does not directly damage or integrate into cellular DNA; and if it has a direct oncogenic effect in causing HCC, it would have to exert it from an extrachromosomal position.[40]

In terms of HBV, HCV and HCC, Donato *et al.*[1] concluded in their meta-analysis that these viruses act through both common and different pathways in the process of hepatocarcinogenesis. It is reasonable to hypothesize that these viruses interact in dually infected persons in a superadditive and sub-, not super-, multiplicative fashion[1] because HBV acts as a cell mutagen and HBV and HCV have overlapping roles in causing both liver cell regeneration and cellular proliferation, leading to clonal expansion. In contrast, the supermultiplicative interaction between acute radiation and HCV that we report suggests that the roles played by these agents overlap to a lesser degree, with acute radiation acting as an agent of mutagenesis or epigenetic change and HCV primarily acting as an agent of cellular proliferation. Lack of evidence of an interaction between HBV and acute radiation in the etiology of HCC suggests that these factors do not play active roles at different steps in hepatocarcinogenesis, perhaps with radiation's role as a mutagen being overshadowed by HBV's strong mutagenic qualities.

Several studies showing a strong link between chronic radiation exposure and liver cancer were conducted in areas of low HCV prevalence, including thorotrast studies in Germany,[41] Denmark[42] and Portugal.[43] Although these studies were limited to liver cancer in general, comparisons with tumor registry data show a statistically significant association between HCC and chronic α-radiation exposure resulting from thorotrast administration.[44] However, chronic and acute exposure to radiation appear to have a different association with liver cirrhosis, a frequent cause of liver cell proliferation. While Andersson *et al.*[45] reported an 11-fold increase in cirrhosis among Danish patients exposed to thorotrast, we found no increased risk of cirrhosis for A bomb irradiation.[46] Thus, while chronic exposure to high radiation doses may cause both genetic alteration and cellular proliferation of hepatocytes leading to HCC, acute radiation exposure at the levels experienced by the A bomb survivors may only cause the first of these events; thus, acute, unlike chronic, radiation exposure may require an additional risk factor for progression to HCC.

In summary, our study suggests that ionizing radiation and HCV infection interact to supermultiplicatively increase the risk of HCC. Based on our results, we conclude that ionizing radiation at the exposure levels studied significantly increases HCC risks in the presence of HCV when cirrhosis is not concurrently detected. Our results fit into a pattern with other studies of interaction in hepatocarcinogenesis, with synergistic or greater than multiplicative interactions in HCC being reported when subjects are exposed both to agents such as radiation that primarily cause genetic alteration and to agents such as HCV and heavy drinking that cause hepatocyte destruction, triggering liver cell regeneration. Our results suggest that persons infected with HCV may be particularly sensitive to radiation exposure and *vice versa.* Future studies of liver irradiation and HCC should take into account HCV infection status.

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

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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).

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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 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 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 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.

Factor[a]	Univariate		Multivariate (adjusted for age and gender)		Multivariate (fully adjusted[c])	
	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 $CD4^+$