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Persistent subclinical inflammation among A-bomb survivors

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Abstract.
Purpose: To investigate the associations between inflammation tests and radiation dose in A-bomb survivors.
Subjects and methods: Subjects were A-bomb survivors who underwent inflammation tests of leukocyte counts, neutrophil counts, erythrocyte sedimentation rate, corrected erythrocyte sedimentation rate, α-1 globulin, α-2 globulin and sialic acid between 1988 and 1992. Associations with radiation dose (DS86) were analyzed by regression analysis and heterogeneity among inflammatory diseases, anaemia at examination, or history of cancer was also tested.
Results: The associations with radiation dose were statistically significant for leukocyte counts ($71.0\,mm^{-3}\,Gy^{-1}$, $p=0.015$), erythrocyte sedimentation rate ($1.58\,mm\,h^{-1}\,Gy^{-1}$, $p=0.0001$), corrected erythrocyte sedimentation rate ($1.14\,mm\,h^{-1}\,Gy^{-1}$, $p=0.0001$), α-1 globulin ($0.0057\,g\,dl^{-1}\,Gy^{-1}$, $p=0.0001$), α-2 globulin ($0.0128\,g\,dl^{-1}\,Gy^{-1}$, $p=0.0001$), and sialic acid ($1.2711\,mg\,dl^{-1}\,Gy^{-1}$, $p=0.0001$) but not for neutrophil counts ($29.9\,mm^{-3}\,Gy^{-1}$, $p=0.17$). Heterogeneity was not statistically significant. Among inflammatory diseases, associations were the strongest for chronic thyroiditis and chronic liver diseases.
Conclusions: This study suggests statistically significant association between inflammation in A-bomb survivors and radiation dose of during 1988–1992. The association might contribute, as an epigenetic and/or bystander effect, to development of several radiation-induced disorders.

1. Introduction

It has been believed that all of the haematological parameters in A-bomb survivors had returned to normal values 2 or 3 years after the event (Snell and Neel 1959). Decades later, the survivors of Hiroshima and Nagasaki exhibited statistically significant associations between radiation dose and levels of inflammatory tests, including leukocytosis, accelerated erythrocyte sedimentation rate (ESR) (Sawada and Kodama 1986), and increased serum concentration of acute phase proteins (Neriishi and Matsuo 1986). In this study, we consider the relationship between laboratory indicators of inflammation and radiation dose in >6000 Hiroshima and Nagasaki A-bomb survivors, 43–47 years after exposure. The specific measures considered are: leukocyte counts, neutrophil counts, ESR, corrected ESR, α-1 globulins (α1-GL), α-2 globulins (α2-GL) and sialic acid. Among those tests, ESR is influenced by a variety of serum components, including acute phase proteins (Sox and Liang 1986), which comprise α1-GL, α2-GL, including α-1 antitrypsin, α-1 acid glycoprotein, haptoglobin, α-2 macroglobulin, C-reactive protein and ceruloplasmin. The concentration of sialic acid, a glycoprotein component released from the surface membrane in the inflammatory process (Stefenelli and Klotz 1985), also correlates well with acute phase proteins (Shiokawa and Kawai 1983). Katz *et al.*, however, reported that in the elderly ESR is a more sensitive and specific index of inflammation than serum levels of C-reactive protein (Katz and Gutman 1989) and acute phase proteins (Katz and Karuza 1990).

2. Subjects and methods

Measurements were made using blood samples obtained during routine biennial examinations of participants in the Radiation Effects Research Foundation's (RERF) Adult Health Study (AHS) programme from March 1988 to February 1992. A total of 7463 people were examined at least once during the period. This represents 70% of surviving AHS cohort members still residing in Hiroshima and Nagasaki. While participation rates depend on age and gender, they do not vary with dose. As described below, analyses were limited to 6304 people with estimated doses and for whom smoking data and at least one inflammatory test result was available. Data for subjects with clinical illnesses diagnosed during the period were analyzed separately from data for those without: (1) persons with inflammatory diseases at the time of examination; (2) persons who had undergone cancer treatment before examination; (3) persons whose cancer(s) was/were detected during the examination period and who underwent inflammatory testing after cancer diagnosis (recent cancer); and (4) persons with anaemia. Clinical conditions and numbers of participants with each condition are shown in table 1. Anaemia was included in this list because it influences ESR, although it is not itself an

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Table 1. Number of subjects by various clinical conditions, March 1988–February 1992.

Clinical conditions	Number of cases[a]
Total subjects	6304
Inflammatory diseases	2923
Chronic liver disease	1030
Infections	972
Osteoarthritis	651
Thyroiditis	274
Rheumatoid arthritis	91
Pneumonia	70
Bronchitis	66
Chronic pancreatitis	49
Chronic glomerulonephritis	27
Tonsillitis	17
Systemic lupus erythematosus	4
Progressive systemic scleroderma	4
Sjogren's disease	4
Ulcerative colitis	3
Polymyositis	2
Dermatomyositis	1
Anemia	389
Cancer in the past[b]	419
Recent cancer[c]	198
No clinical inflammation	3074

[a] Since subjects may have more than one inflammatory disease, the numbers total more than 6304.
[b] Those who had cancer treatment before March 1988.
[c] Those whose cancer was detected between March 1988 and February 1992 and who underwent inflammatory tests at RERF after cancer detection.

inflammatory condition. Persons with inflammatory conditions were identified by the International Code for Diseases from the AHS program database. A relatively large number of cases of thyroiditis were diagnosed by the presence of thyroid antibodies detected in a special thyroid study conducted in Nagasaki participants between 1984 and 1987.

2.1. *Laboratory tests*

Blood samples were divided into two portions. Anticoagulants were added to one portion. Blood aliquots with anticoagulants were used for ESR and complete blood counts, while blood aliquots without anticoagulants were used for protein electrophoresis and sialic acid analysis. Leukocyte counts were obtained with a blood cell counter (Towa Sysmex CC180, Tokyo, Japan). ESR was measured by the Wintrobe method (Sox and Liang 1986) and corrected by a reading of the Wintrobe diagram based on volumes of packed red cells. Blood collection methods differed for the two cities with a greater time delay between blood drawing and testing in Hiroshima. α-Globulin levels were calculated by electrophoretic fractionation of serum protein (Separax, Fujifilm, Ltd, Tokyo, Japan) and quantification with a densitometer (Olympus AES310, Tokyo, Japan). Sialic acid was assayed with a kit (Determiner SA, Kyowa Medics, Ltd, Tokyo, Japan) and an automatic analyser (Hitachi 7050, Tokyo, Japan).

2.2. *Dosimetry*

Estimated bone marrow doses were based on the DS86 dosimetry system, which calculates a free-in-air estimate for the subject's reported location and is adjusted to reflect shielding information. The unit of absorbed ionizing radiation for both γ-rays and neutrons is the gray (Gy). Doses >4 Gy were truncated to 4 Gy because the inclusion or exclusion of those with >4 Gy did not affect the results.

2.3. *Smoking*

Data on the number of cigarettes smoked per day at the time of examination were obtained from interviews conducted by nurses during the routine AHS clinical examination.

2.4. *Statistical analysis*

Among 6304 individual subjects, 4401 (69.8%) had two sets of measurements (one taken between 1988 and 1990, the other in 1990–1992), 1492 (23.7%) had one set, and 411 (6.5%) had more than two sets. Correlation between the two sets of measurements in each inflammatory test was significant: leukocyte counts ($r=0.68$, $p=0.0001$, paired comparison $p=0.61$), neutrophil counts ($r=0.55$, $p=0.019$, paired comparison $p=0.09$), ESR ($r=0.80$, $p=0.0001$, paired comparison $p=0.0001$), CESR ($r=0.77$, $p=0.0001$, paired comparison $p=0.0001$), α1-GL ($r=0.27$, $p=0.0001$, paired comparison $p=0.0001$), α2-GL ($r=0.64$, $p=0.0001$, paired comparison $p=0.0002$), and sialic acid ($r=0.61$, $p=0.0001$, paired comparison $p=0.0018$). An average of the measurements was used for persons with multiple measurements. Least-square regression, which would produce conservative Wald tests in any of variables, led to significant differences in terms of dose-effects. The adjustment of the variance-in-regression (weighted regression) was not applied. The numbers of subjects in the analyses varied slightly due to missing data for some specific tests. The multivariate test (Wilk's Lambda) for radiation dose-effects was significant ($p=0.0001$). We used regression analysis of non-transformed data to estimate trends in inflammation measurements by city (Hiroshima relative to Nagasaki), age (by 10-year group), sex (male or

female), smoking (0–9, 10–19, 20–29, 30+ cigarettes per day), and radiation dose (Gy). The results of regression analysis were the same regardless of whether or not log-transformation was applied. We added the quadratic dose term to the regression model and the results indicated that the quadratic dose–response relationship fitted for leukocytes, neutrophils, and α-2 globulins, while the linear model was appropriate for the other tests. Our main purpose in this study was to see whether inflammation test levels had increased and since the shapes of the dose–response relationships are a secondary aspect in this paper, we have analyzed the data only with a linear model that included five main variables: city, sex, age, smoking and DS86 radiation dose estimate.

3. Results

Table 2 shows subject profiles, including mean age, number of cigarettes per day and mean radiation dose among the 6304 survivors. Table 3 shows average values of inflammation measurements by sex and radiation exposure categories. Exposed persons had higher levels of all inflammatory scores, although the magnitude of differences varied by test. Leukocyte and neutrophil counts increased more in men than in women, whereas ESR increased more in women than men.

After allowing for the influence of city, age, sex, and smoking, regression analysis indicate statistically significant associations with radiation dose for leukocyte counts ($71.0\,mm^{-3}\ Gy^{-1}$, $p=0.00151$), ESR ($1.57\,mm\ h^{-1}\ Gy^{-1}$, $p=0.0001$), CESR ($1.14\,mm\ h^{-1}\ Gy^{-1}$, $p=0.0001$), α1-GL ($0.0057\,g\ dl^{-1}\ Gy^{-1}$, $p=0.0001$), α2-GL ($0.0128\,g\ dl^{-1}\ Gy^{-1}$, $p=0.0001$), and sialic acid ($1.2711\,mg\ dl^{-1}\ Gy^{-1}$, $p=0.0001$) but not for neutrophil counts ($29.9\,mm^{-3}\ Gy^{-1}$, $p=0.17$) (table 4). Interactions between dose and inflammatory disease were suggested only for sialic acid ($p=0.044$), but not for leukocyte counts ($p=0.37$), neutrophil counts ($p=0.24$), ESR ($p=0.94$), CESR ($p=0.57$), α1-GL ($p=0.098$) or α2-GL ($p=0.059$) (table 4). Major inflammatory diseases were tested for dose–response, and chronic thyroiditis and chronic liver diseases were found to be the principal diseases showing significant association with radiation dose. After allowing for the influence of covariables, regression analyses for chronic thyroiditis showed statistically significant relationships with radiation dose for leukocyte counts ($340.7\,mm^{-3}\ Gy^{-1}$, $p=0.014$), neutrophil counts ($195.0\,mm^{-3}\ Gy^{-1}$, $p=0.043$), ESR ($3.95\,mm\ h^{-1}\ Gy^{-1}$, $p=0.0003$), CESR ($2.24\,mm\ h^{-1}\ Gy^{-1}$, $p=0.0106$), α1-GL ($0.0074\,g\ dl^{-1}\ Gy^{-1}$, $p=0.052$) and α2-GL ($-0.0026\,g\ dl^{-1}\ Gy^{-1}$, $p=0.76$) but not for sialic acid ($0.689\,mg\ dl^{-1}\ Gy^{-1}$, $p=0.41$).

Table 5 shows regression analyses for all inflammatory tests by individual covariable. The covariable indicator for inflammatory disease and the interaction between dose and indicator variable were excluded from the model depending on the significance in the first model. 'Intercept' estimates represent average test responses in female Nagasaki non-smokers at 60 years of age. The city and smoking effects on ESR were the reverse of those seen for other inflammatory tests. There is a significant negative influence of smoking on ESR but not CESR and the remaining inflammatory test measurements show a positive effect. For α1-GL and sialic acid, relationship to 1 Gy radiation exposure is roughly the same as a 10-year rise in age or as smoking ten cigarettes per day. The relative magnitude of the radiation association seen for neutrophil or leukocyte counts is much smaller, with a 1 Gy radiation dose showing only about one-third of the impact of a 10-year age change or smoking 10 cigarettes per day.

Regression analysis limited to non-smokers also showed a significant relationship with radiation dose for neutrophil count ($p=0.0063$), ESR ($p=0.0005$), CESR ($p=0.0002$), α2-GL ($p=0.011$) and sialic acid ($p=0.0001$), but not for leukocyte counts and α1-GL (data not shown).

To provide some perspective on the size of these radiation dose relationships, we have plotted for each set of inflammatory test measurements the test results by dose adjusted for the effects of city, sex, age and smoking (figure 1A–G).

4. Discussion

This study suggests that dose-related inflammatory processes are operating in the survivors with or without the presence of clinically detectable inflammatory disorders. While selection bias or confounding factors, such as socio-economic status, lifestyle, or genetic factors, might contribute to this finding, no associations with known variables (city of residence, sex, age, smoking) were sufficient to have produced spurious results. The presence of inflammatory disorders might also have confounded our results. No such effects were found, however, when heterogeneity among persons with and without clinically detectable inflammatory disorders was tested. Chronic thyroiditis and chronic liver diseases were found to be the principal diseases showing significant relationships to radiation dose. Interestingly, no prior association between prevalence of chronic thyroiditis in A-bomb survivors and radiation dose has been reported (Nagataki and Shibata 1994), suggesting

Table 2. Subject profiles, including mean age, number of cigarettes per day and mean dose (Gy).

	Male			Female			Total		
	Hiroshima	Nagasaki	Total	Hiroshima	Nagasaki	Total	Hiroshima	Nagasaki	Total
Total									
No. subjects	1378	811	2189	2820	1295	4115	4198	2106	6304
Mean age	60.9	60.8	60.9	65.4	63.1	64.7	63.9	62.2	63.4
SD	12.3	11.0	11.8	11.8	10.4	11.5	12.2	10.7	11.7
No. cigarettes	10.4	10.3	10.3	1.1	0.8	1.0	4.2	4.5	4.3
SD	13.0	12.9	13.0	4.1	3.4	3.9	9.3	9.6	9.4
Mean dose (Gy)	0.38	0.44	0.40	0.34	0.41	0.36	0.35	0.42	0.38
SD	0.70	0.74	0.71	0.62	0.65	0.63	0.65	0.69	0.66
0–0.01 Gy									
No. subjects	587	415	1002	1168	604	1772	1755	1019	2774
Mean age	60.8	61.7	61.2	64.9	62.8	642	63.7	62.4	63.1
SD	12.9	11.5	12.3	12.3	10.3	11.7	12.7	10.8	12.0
No. cigarettes	10.0	10.2	10.1	1.0	0.9	1.0	4.0	4.7	4.2
SD	12.7	13.2	12.9	4.0	3.5	3.8	9.1	9.9	9.4
Mean dose (Gy)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
SD	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.01–0.999 Gy									
No. subjects	614	264	878	1369	520	1889	1983	784	2767
Mean age	61.3	59.5	60.7	66.0	63.6	65.3	64.5	62.2	63.9
SD	12.1	10.3	11.6	11.6	10.8	11.4	12.0	108	11.7
No. cigarettes	10.9	10.4	10.7	1.3	1.0	1.2	4.3	4.1	4.2
SD	13.3	13.0	13.2	4.3	3.7	4.2	9.4	9.3	9.3
Mean dose (Gy)	0.32	0.47	0.37	0.32	0.48	0.36	0.32	0.48	0.36
SD	0.27	0.29	0.28	0.26	0.29	0.28	0.26	0.29	0.28
1.000–1.999 Gy									
No. subjects	129	107	236	187	142	329	316	249	565
Mean age	60.4	61.8	61.0	65.5	63.0	64.4	63.4	62.5	63.0
SD	11.0	10.6	10.8	11.8	9.2	10.8	11.7	9.8	11.0
No. cigarettes	10.3	10.0	10.1	1.3	0.3	0.8	4.9	4.4	4.7
SD	13.3	12.2	12.8	4.1	1.6	3.3	10.1	9.4	9.8
Mean dose (Gy)	1.40	1.37	1.39	1.40	1.34	1.37	1.40	1.35	1.38
SD	0.27	0.29	0.28	0.26	0.26	0.26	0.26	0.27	0.27
> 2.0 Gy									
No. subjects	48	25	73	96	29	125	144	54	198
Mean age	58.2	55.5	57.2	63.0	61.3	62.3	61.4	58.6	60.6
SD	8.7	8.1	8.5	9.5	11.0	9.8	9.5	10.1	9.7
No. cigarettes	9.3	10.6	9.7	0.8	0.0	0.6	3.7	4.9	4.0
SD	11.5	11.6	11.4	2.7	0.0	2.7	8.0	9.4	8.4
Mean dose (Gy)	3.08	3.38	3.18	2.80	3.34	2.93	2.89	3.36	3.02
SD	1.01	1.02	1.02	0.83	0.97	0.89	0.90	0.98	0.95

that radiation-induced inflammation may occur preferentially in persons predisposed to having thyroid auto-antibodies. Possibly undetected cases of chronic thyroiditis may have contributed to an increase in levels of inflammatory tests.

Effects of city and smoking on ESR were the reverse of those seen with other inflammatory test measurements. The influence of city residence is not clear. It may relate to socio-economic conditions, undetected dose differences, and/or unknown factors. One possibility is the different blood collection methods used in the two cities. The greater time delay between blood drawing and testing in Hiroshima may have caused an artificial decrease in the ESR measurement (Sox and Liang 1986). The results for other inflammatory tests, which agree with each other, may represent a true difference between the cities. The negative effect of smoking on ESR is also not clear. It could be a matter of chance because CESR results are not significantly different while other inflammatory test measurements show positive relationships. Although the factors considered here, including radiation, may explain only a fraction of the inflammation measurement increases, relatively large doses of radiation ($\sim$ 1 Gy) appear to have effects compatible in some cases with those seen for

Table 3. Mean values of inflammatory tests by exposure groups in A-bomb survivors between 1988 and 1992.

	0–0.01 Gy		0.01–0.999 Gy		1.000–1.999 Gy		> 2.000 Gy		Total	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Leukocytes mm^{-3}										
Male	6483.8	1733.1	6528.1	1856.4	6445.7	1721.2	6750.7	1687.7	6506.5	1780.5
Female	5525.6	1331.0	5605.9	1450.5	5607.2	1357.8	5847.5	1474.1	5578.8	1394.5
Total	5872.0	1558.2	5898.9	1647.4	5957.4	1574.2	6180.5	1612.3	5901.2	1601.7
Neutrophils mm^{-3}										
Male	3646.7	1274.8	3670.0	1349.9	3654.8	1251.8	3656.8	1196.7	3657.2	1299.8
Female	3099.2	1002.3	3117.4	1067.3	3119.8	983.2	3266.0	1077.9	3114.3	1033.5
Total	3297.1	1139.2	3293.0	1192.3	3343.3	1133.5	3409.9	1135.9	3303.0	1162.2
Erythrocyte sedimentation rate ($mm\ h^{-1}$)										
Male	16.0	11.2	16.2	11.3	18.8	11.6	18.2	10.8	16.5	11.3
Female	25.2	11.2	26.0	11.5	28.4	11.6	28.8	12.3	25.9	11.5
Total	21.8	12.1	22.9	12.3	24.4	12.5	24.9	12.8	22.6	12.3
Corrected erythrocyte sedimentation rate[a] ($mm\ h^{-1}$)										
Male	12.2	8.6	12.2	8.9	14.1	9.2	14.3	8.3	12.5	8.8
Female	20.7	9.6	21.2	9.5	23.3	9.3	22.6	10.3	21.2	9.6
Total	17.6	10.1	18.3	10.2	19.4	10.3	19.5	10.4	18.2	10.2
Alpha-1 globulus ($g\ dl^{-1}$)										
Male	0.251	0.044	0.254	0.046	0.258	0.047	0.269	0.043	0.254	0.045
Female	0.249	0.043	0.253	0.045	0.254	0.047	0.263	0.050	0.252	0.044
Total	0.250	0.043	0.253	0.045	0.256	0.047	0.265	0.047	0.252	0.045
Alpha-2 globulus ($g\ dl^{-1}$)										
Male	0.688	0.107	0.691	0.110	0.694	0.101	0.709	0.101	0.690	0.107
Female	0.713	0.101	0.722	0.105	0.720	0.097	0.750	0.116	0.719	0.103
Total	0.704	0.104	0.712	0.107	0.709	0.099	0.735	0.112	0.709	0.105
Sialic acid ($mg\ dl^{-1}$)										
Male	68.1	9.2	68.0	9.2	69.4	9.5	70.8	7.9	68.3	9.2
Female	67.8	8.8	68.4	9.2	68.8	8.4	70.6	8.5	68.2	9.0
Total	67.9	8.9	68.3	9.2	69.0	8.9	70.7	8.3	68.2	9.1

[a] Corrected by a reading of Wintrobe diagram based on volumes of packed red cells.

Table 4. Regression analysis for radiation effects of inflammatory tests in A-bomb survivors and the interactions between dose and inflammatory diseases after allowing for effects of age, sex, city and smoking.

Inflammatory tests	Parameter estimate[a]	SE	Probability of *t*-test for dose	Probability of *t*-test for interaction between dose and inflammatory diseases	Probability of *t*-test for indicator of inflammatory disease
Leukocyte	71.0	29.2	0.0151	0.3717	0.4756
Neutrophil	29.9	21.9	0.1729	0.2387	0.0913
Erythrocyte Sedimentation Rate (ESR)	1.57	0.21	0.0001	0.9356	0.0001
Corrected ESR	1.14	0.17	0.0001	0.5712	0.0001
Alpha-1 globulins	0.0057	0.0012	0.0001	0.0983	0.0139
Alpha-2 globulins	0.0128	0.0028	0.0001	0.0590	0.0001
Sialic acid	1.2711	0.2442	0.0001	0.0438	0.0001

[a] Units are $mm^3\ Gy^{-1}$ in leukocyte and neutrophil, $mm\ h^{-1}\ Gy^{-1}$ in ESR and corrected ESR, $g\ dl^{-1}\ Gy^{-1}$ in alpha-1 globulins and alpha-2 globulins, and $mg\ dl^{-1}\ Gy^{-1}$ in sialic acid.

other factors generally accepted to affect such measurements. In particular, for α1-GL and sialic acid, the effect of 1 Gy radiation exposure is roughly the same as the impact of a 10-year increase in age or smoking 10 cigarettes per day. However, the relative magnitude of the radiation effect seen for neutrophil or leukocyte counts is much smaller, 1 Gy having about one-third the impact of a 10-year age change or smoking 10 cigarettes per day.

The mechanism by which the association between

Table 5. Regression analysis of covariables in inflammatory tests in A-bomb survivors between 1988 and 1992.

Variable	Leukocytes (mm^{-3})	Neutrophils (mm^{-3})	Erythrocyte sedimentation rate ($mm\ h^{-1}$)	Corrected erythrocyte sedimentation rate[a] ($mm\ h^{-1}$)	Alpha-1 globulins ($g\ dl^{-1}$)	Alpha-2 globulins ($g\ dl^{-1}$)	Sialic acid ($mg\ dl^{-1}$)
R^2 =	0.14	0.09	0.27	0.25	0.08	0.09	0.05
Intercept	5470.95 ± 38.27[b]	3129.37 ± 31.13	23.71 ± 0.29	20.50 ± 0.25	0.231 ± 0.0012	0.6732 ± 0.0029	66.00 ± 0.25
Indicator of inflammatory disease	−(0.4756)[c]	−74.51 ± 28.20(0.0082)	2.21 ± 0.27(0.0001)	1.25 ± 0.22(0.0001)	0.00310 ± 0.00169(0.0139)	0.0145 ± 0.0030(0.0001)	1.0059 ± 0.2589(0.0001)
Age (per decade)	−29.9 ± 16.48(0.0697)	35.62 ± 12.39(0.0041)	3.48 ± 0.12(0.0001)	2.33 ± 0.10(0.0001)	0.00659 ± 0.00048(0.0001)	0.0173 ± 0.0011(0.0001)	1.587 ± 0.098(0.0001)
Male	461.37 ± 44.60(0.0001)	304.59 ± 33.46(0.0001)	−7.54 ± 0.31(0.0001)	−7.74 ± 0.27(0.0001)	0.000294 ± 0.00129(0.8198)	−0.0278 ± 0.00303(0.0001)	−0.011 ± 0.265(0.9670)
Hiroshima	65.48 ± 36.81(0.1000)	−51.54 ± 29.87(0.0845)	−1.43 ± 0.28(0.0001)	−2.05 ± 0.24(0.0001)	0.01960 ± 0.001150(0.0001)	0.03840 ± 0.0027(0.0001)	0.826 ± 0.237(0.0005)
Smoking (per 10 cigarettes per day)	490.60 ± 23.00(0.0001)	265.40 ± 17.25(0.0001)	−0.71 ± 0.16(0.0055)	−0.180 ± 0.14(0.1886)	0.005618 ± 0.00066(0.0001)	0.00888 ± 0.00156(0.0001)	0.7988 ± 0.137(0.0001)
Dose (per Gy)	70.00 ± 29.22(0.0151)	29.89 ± 21.93(0.1729)	1.58 ± 0.21(0.0001)	1.14 ± 0.17(0.0001)	0.00573 ± 0.00118(0.0001)	0.0128 ± 0.0028(0.0001)	1.2711 ± 0.244(0.0001)
Interaction between dose and inflammatory disease	−(0.3717)	−(0.2387)	−(0.9356)	−(0.5712)	−0.002787 ± 0.00168(0.0983)	−0.0074 ± 0.0040(0.059)	−0.7001 ± 0.347(0.0438)

[a] Corrected by a reading of Wintrobe diagram based on volumes of packed red cells.

[b] Intercept estimates average response in female Nagasaki nonsmokers at 60 years of age. Parameter estimates for city, age, smoking, sex and dose represent change per 10-year increase in age, difference between males and females, difference between Hiroshima and Nagasaki, change per 10 cigarettes per day, change per Gy increase of radiation dose.

[c] Numbers in the parentheses are *p*. Interactions, city by sex, smoking by sex, and city by age were significant commonly in 4, 4, 3 inflammatory tests, respectively (not shown on the table).

laboratory indicators of inflammation and radiation dose may persist long after irradiation is not clear. Johnston and Pieboeuf (1996) reported a persistent alteration of inflammatory cytokine mRNA expression in fibrosis-sensitive mice after thoracic irradiation, which suggests that inflammatory cytokine mRNA could play a role in our findings. In our study, evidence of an increase in leukocyte counts in A-bomb survivors suggests a role for inflammatory cytokine stimulation, exceeding that of stem-cell damage, which would have resulted in a decrease in leukocyte count. Sakaguchi and Miyai (1994) reported that radiation may induce autoimmunity. Since A-bomb survivors are known to have impaired T-cell immunity (Akiyama and Kusunoki 1991, Kusunoki and Kyoizumi 1994, 1998), the presence of auto-antibodies, together with T-cell immune impairment, might have led to persistent inflammation governed by the extent of T-cell immune impairment. Preliminary analyses suggest that a decrease in CD4+ T-cells is significantly associated with increase of inflammatory test values (data not shown). Emerit and Levy (1994) reported that serum components capable of inducing chromosome breakage, detected in cases with autoimmune diseases (Emerit 1980), were present in Chernobyl recovery workers several years after exposure (Emerit and Levy 1994). The increase in inflammation test levels, observed in chronic thyroiditis and chronic liver disease, as well as in persons without clinical inflammatory conditions, appear to indicate a general intensification of inflammation, probably via immunological damage in accordance with radiation dose.

Of possible relevance to our findings of persistent inflammation in A-bomb survivors may be recent reports suggesting an inflammation mechanism underlying cardiovascular disease (Ross 1999) and hypercalcaemia (Devlin and Reddy 1998). Radiation-induced enhancement of inflammatory reactions might contribute, as an epigenetic and/or bystander effect, to development of such disorders in A-bomb survivors. Dose-related increases in certain non-cancer diseases in A-bomb survivors, including cardiovascular diseases, lend some credence to this idea (Robertson and Shimizu 1979, Shimizu and Pierce 1999).

In conclusion, our data suggest that an increase in levels of inflammatory tests between 1988 and 1992

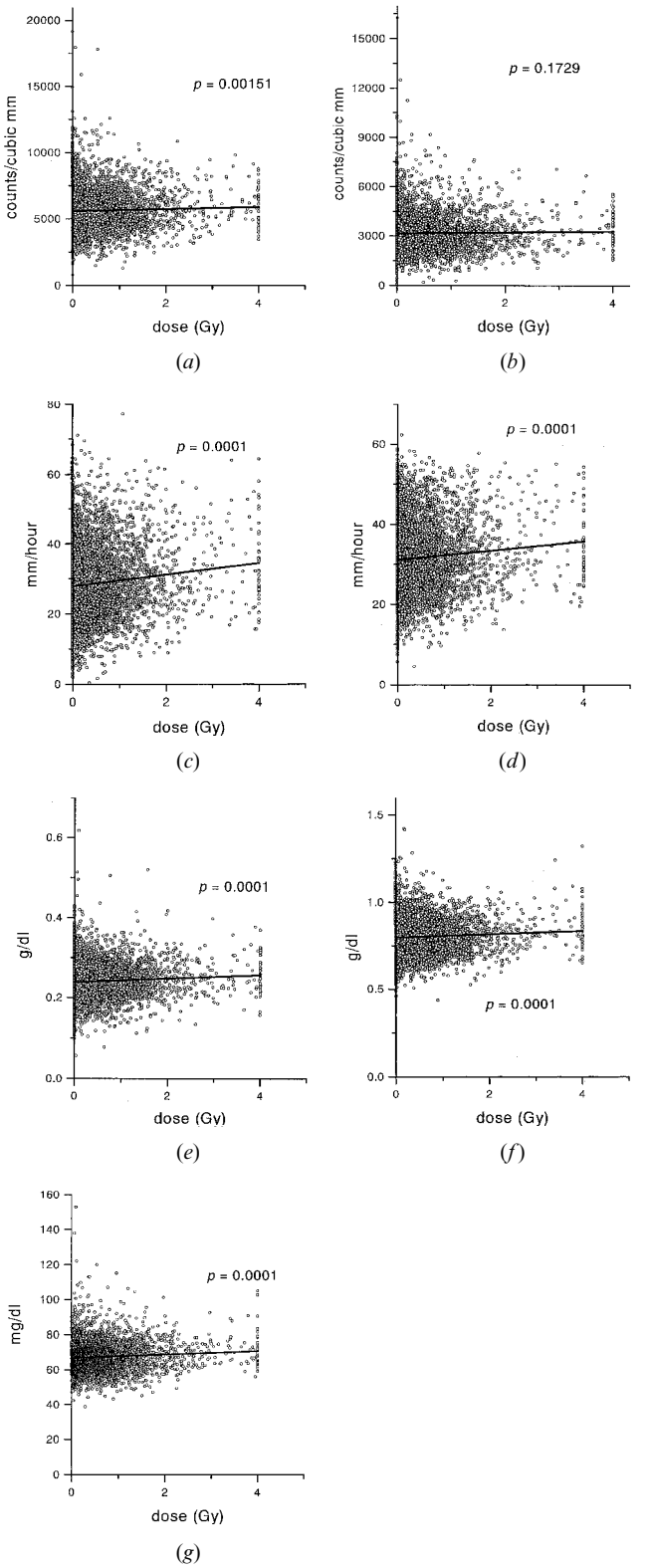

Figure 1. Dose–response in inflammatory test variables adjusted for city, age, sex and smoking: (A) leukocyte counts ($71.0\,mm^{-3}\ Gy^{-1}$, $p=0.015$), (B) neutrophil counts ($29.9\,mm^{-3}\ Gy^{-1}$, $p=0.17$), (C) erythrocyte sedimentation rate ($1.58\,mm\ h^{-1}\ Gy^{-1}$, $p=0.0001$), (D) corrected erythrocyte sedimentation rate ($1.14\,mm\ h^{-1}\ Gy^{-1}$, $p=0.0001$), (E) α-1 globulins ($0.0057\ g\ dl^{-1}\ Gy^{-1}$, $p=0.0001$), (F) α-2 globulins ($0.0128\ g\ dl^{-1}\ Gy^{-1}$, $p=0.0001$) and (G) sialic acid ($1.281\ mg\ dl^{-1}\ Gy^{-1}$, $p=0.0001$).

is associated with A-bomb radiation among A-bomb survivors. Such associations were seen regardless of the presence of clinically detectable inflammatory disorders. Further studies are needed to explore possible mechanisms that may lead to inflammation persisting long after radiation exposure.

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References

AKIYAMA, M. and KUSUNOKI, Y., 1991, Overview of immunological studies on A-bomb survivors. *Journal of Radiation Research*, **32 (suppl.),** 301–309.

DEVLIN, R. D. and REDDY, S. V., 1998, IL-6 mediates the effects of IL-1 or TNF, but not PTHrP or 1,25(OH)2D3, on osteoclast-like cell formation in normal human bone marrow cultures. *Journal of Bone Mineral Research*, **13,** 393–399.

EMERIT, I., 1980, Chromosomal instability in collagen disease. *Z Rheumatology*, **39,** 84–90.

EMERIT, I., 1994, Reactive oxygen species, chromosome mutation and cancer: possible role of clastogenic factors in carcinogenesis. *Free Radical Biological Medicine*, **16,** 99–109.

EMERIT, I. and LEVY, A., 1994, Transferable clastogenic activity in plasma from persons exposed as salvage personnel of the Chernobyl reactor. *Journal of Cancer Research and Clinical Oncology*, **120,** 558–561.

JOHNSTON, C. J. and PIEBOEUF, B., 1996, Early and persistent alterations in the expression of interleukin-1 α, interleukin-1 β and tumor necrosis factor α mRNA levels in fibrosis-resistant and -sensitive mice after thoracic irradiation. *Radiation Research*, **145,** 762–767.

KATZ, P. R. and GUTMAN, S. I., 1989, Erythrocyte sedimentation rate and C-reactive protein compared in the elderly. *Clinical Chemistry*, **35,** 466–468.

KATZ, P. R. and KARUZA, J., 1990, A comparison between erythrocyte sedimentation rate (ESR) and selected acute-phase proteins in the elderly. *American Journal of Clinical Pathology*, **94,** 637–640.

KUSUNOKI, Y. and KYOIZUMI, S, 1994, Increased frequency of $CD4^-CD8^-$ cells bearing T-cell receptor alpha beta chains in peripheral blood of atomic bomb survivors exposed to high dose. *Radiation Research*, **139,** 67–72.

KUSUNOKI, Y. and KYOIZUMI, S., 1998, Flow cytometry measurements of subsets of T, B and NK cells in peripheral blood lymphocytes of atomic bomb survivors. *Radiation Research*, **151,** 227–236.

NAGATAKI, S. and SHIBATA, Y., 1994, Thyroid diseases among A-bomb survivors in Nagasaki. *Journal of the American Medical Association*, **272,** 364–370.

NERIISHI, K. and MATSUO, N. 1986, Relationship between radiation exposure and serum protein alpha and beta globulin fractions. *Nagasaki Medical Journal*, **61,** 449–454.

ROBERTSON, T. L. and SHIMIZU, Y., 1979, *Incidence of Stroke and Coronary Heart Disease in Atomic Bomb Survivors Living in Hiroshima and Nagasaki, 1958–74*. RERF TR 12-79 (Hiroshima and Nagasaki: RERF).

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

SAKAGUCHI, N. and MIYAI, K., 1994, Ionizing radiation and autoimmunity. Induction of autoimmune disease in mice by high dose fractionated total lymphoid irradiation and its prevention by inoculating normal T cells. *Journal of Immunology*, **152,** 2586–2595.

SAWADA, H. and KODAMA, K., 1986, *Adult Health Study Report 6. Results of Six Examination Cycles, 1968–1980*. RERF TR 3-86 (Hiroshima and Nagasaki: RERF).

SHIMIZU, Y. and PIERCE, D. A., 1999, Studies of the mortality of A-bomb survivors. Report 12, Part II. Noncancer mortality: 1950–1990. *Radiation Research*, **152,** 374–389.

SHIOKAWA, Y. and KAWAI, T., 1983, Methods for sialic acid and its clinical application. *Japanese Journal of Clinical Pathology*, **S54,** 1–209.

SNELL, F. M. and NEEL, J. V., 1959, *Hematologic Studies in Hiroshima and a Control City 2 Years After the Atomic Bomb*. ABCC/RERF TR 27-A-59 (Hiroshima and Nagasaki: RERF).

SOX, H. C. and LIANG, M. H., 1986, The erythrocyte sedimentation rate: Guidelines for rational use. *Annals of Internal Medicine*, **104,** 515–523.

STEFENELLI, N. and KLOTZ, H., 1985, Serum sialic acid in malignant tumours, bacterial infections and chronic liver diseases. *Journal of Cancer Research Clinical Oncology*, **109,** 55–59.