

T-Cell Immunosenescence and Inflammatory Response in Atomic Bomb Survivors

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In this paper we summarize the long-term effects of A-bomb radiation on the T-cell system and discuss the possible involvement of attenuated T-cell immunity in the disease development observed in A-bomb survivors. Our previous observations on such effects include impaired mitogen-dependent proliferation and IL-2 production, decreases in naive T-cell populations, and increased proportions of anergic and functionally weak memory CD4 T-cell subsets. In addition, we recently found a radiation dose-dependent increase in the percentages of CD25⁺/CD127⁻ regulatory T cells in the CD4 T-cell population of the survivors. All these effects of radiation on T-cell immunity resemble effects of aging on the immune system, suggesting that ionizing radiation might direct the T-cell system toward a compromised phenotype and thereby might contribute to an enhanced immunosenescence. Furthermore, there are inverse, significant associations between plasma levels of inflammatory cytokines and the relative number of naive CD4 T cells, also suggesting that the elevated levels of inflammatory markers found in A-bomb survivors can be ascribed in part to T-cell immunosenescence. We suggest that radiation-induced T-cell immunosenescence may result in activation of inflammatory responses and may be partly involved in the development of aging-associated and inflammation-related diseases frequently observed in A-bomb survivors. © 2010 by Radiation Research Society

INTRODUCTION

Epidemiological studies of A-bomb survivors have suggested a relationship between radiation dose and the mortality or morbidity rates for various noncancer diseases (1, 2). Risk estimates made for the years from 1950 to 1997 were found to be elevated for death from all solid cancers combined and also for death from all noncancer diseases combined, with excess relative risks (ERRs) per Gy of 0.47

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and 0.14, respectively (1). While the estimated ERR for noncancer disease death is clearly low, the death toll has risen to about 32,000, representing approximately 70% of the total recorded deaths in the cohort (1). Mechanisms for radiation-related cancer, although not totally understood, are much clearer than those for radiation-related noncancer diseases, about which almost nothing is known. An interesting hypothesis is that radiation effects on the immune system may be involved in part in radiation-related diseases, especially for noncancer diseases.

Advancing age is accompanied by a variety of alterations in the immune system, many of which will tend to increase the susceptibility of elderly people to a wide range of diseases. Thus, for example, age-dependent decreases in T-cell numbers and/or function are almost certain to lead to increases in vulnerability to disease-causing pathogens as well as to several adverse manifestations of chronic inflammation. In A-bomb survivors, dose-dependent increases in morbidity have been associated with a variety of inflammatory diseases, such as chronic liver diseases, thyroid diseases and heart diseases (2). To gain further insights into the mechanisms of radiation-related noncancer diseases, we have been focusing on aging- and radiation-related alterations in T-cell immunity. In this paper, we first summarize the long-term effects of A-bomb radiation on the T-cell system, including current study results on regulatory T cells, and then we discuss a possible involvement of attenuated T-cell immunity in the development of diseases frequently observed in A-bomb survivors.

MATERIALS AND METHODS

Blood Donors

A total of 1,035 study subjects were randomly selected from Hiroshima participants in the Adult Health Study (AHS) at the Radiation Effects Research Foundation (RERF) (3). For the present study, blood samples from the subjects were obtained with informed consent when they participated in the AHS health examination program between 2006 and 2008. The study protocol has been approved by the Human Investigation Committee of RERF (the RERF Institutional Review Board). Cancer prevalence within the study subjects by dose

TABLE 1
Characterization of the Study Population

Dose (Gy)	Age (years) ^b						Total
	<70		70–79		80+		
	Male	Female	Male	Female	Male	Female	
<0.005 ^a	29	26	73	102	14	97	341
0.005–0.5	11	34	62	86	22	133	348
0.5–1.0	18	23	19	41	19	48	168
1.0–4.0	28	32	29	40	17	32	178
Total	86	115	183	269	72	310	1,035

^a Individuals in this dose category were exposed at distances in excess of 3 km from the hypocenter and hence received doses that are equivalent to zero.

^b Age at the time of the examinations that were conducted between 2006 and 2008.

category was 14% at <0.005 Gy, 16% at 0.005–0.5 Gy, 22% at 0.5–1.0 Gy, and 24% at ≥1.0 Gy and tended to be higher in survivors exposed to higher doses, in accord with a recent observation in the AHS population (4). The age-, gender- and radiation dose-specific distributions of the 1,035 study subjects are listed in Table 1. Radiation doses are based on Dosimetry System 02 (DS02) estimates (5).

Flow Cytometry

Mononuclear cell fractions separated by the Ficoll-Hypaque gradient technique were analyzed by three-color flow cytometry using a FACScan flow cytometer (BD Biosciences, San Jose, CA) as described previously (6). For assessment of regulatory T (Treg) cells, approximately 100,000 mononuclear cells were stained with Alexa Fluor® 488-labeled CD25 mAb (Serotec Ltd, Oxford, UK), PE-labeled anti-CD127 mAb (BD Biosciences), and PerCP-labeled CD4 mAb (BD Biosciences). Analyses of naïve and effector/memory cell subsets in the CD4 T-cell population involved staining mononuclear cell fractions with FITC-labeled anti-CD45RA mAb (Beckman Coulter, Inc., Fullerton, CA), PE-labeled anti-CD62L mAb (BD Biosciences), and PerCP-labeled CD4 mAb.

TNF- α Measurement

Of the 1,035 subjects examined for their T-cell subsets, we selected 69 subjects whose DS02 doses exceeded 1 Gy as well as 86 age- and gender-matched subjects who were exposed to less than 5 mGy and analyzed their plasma TNF- α levels. Since the sample selection was originally made for the purpose of examining associations between inflammatory responses and mutant frequency at the glycophorin A locus in erythrocytes (4), all subjects were all heterozygous for blood-type MN at that locus. TNF- α levels were analyzed using a highly sensitive enzyme-linked immunosorbent assay kit (Quantikine HS, R&D systems, Minneapolis, MN). The minimum detectable concentration was 0.05 pg/ml.

Data Analysis

Associations of the percentage (Y) of each CD4 T-cell subpopulation with age at time of examination (age), gender ($gender$) and radiation dose ($dose$) were analyzed using a multiple regression model (7). This model assumes that the percentage of each T-cell subpopulation, or level of TNF- α , is related to each variable in an exponential manner with adjustment for age, gender and other parameters (V) measured for the same individuals:

$$\log Y = \alpha + \beta_1 \times age + \beta_2 \times gender + \beta_3 \times dose + \beta_4 \times V,$$

where $gender$ is an indicator of female sex, i.e., $gender = 0$ for male

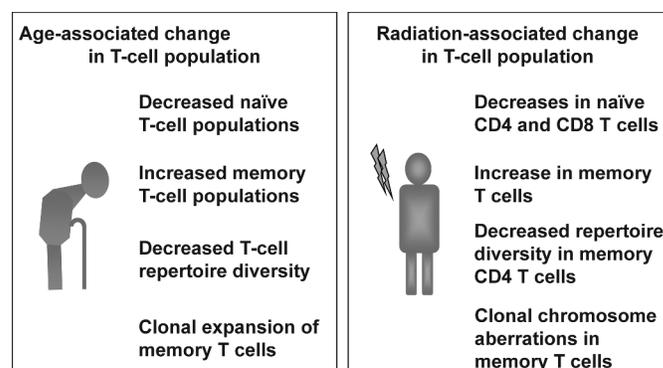


FIG. 1. Age- and radiation-associated alterations in the T-cell system in humans. Left panel: An outline of aging of the T-cell system from a review by Weng (8). Right panel: Alterations in the T-cell systems we have observed in association with radiation exposure among A-bomb survivors (6, 9–12).

and $gender = 1$ for female, and $dose$ is radiation dose in grays. The α is a constant term, and β_1 , β_2 , β_3 and β_4 are regression coefficients for variables to be estimated. The age term was subtracted by 70 years so that α corresponds to log-transformed percentage of CD4 T-cell subset, i.e., the subset percentage is calculated to be e^α (= exponential [α]), for nonirradiated males at 70 years of age. The percentage change of subset percentage was estimated to be $100(e^{10\beta_1} - 1)$ per 10 years increment of age and $100(e^{\beta_3} - 1)$ per 1 Gy radiation dose. All statistical analyses were carried out using the SAS program (SAS Institute Inc., Cary, NC).

RESULTS AND DISCUSSION

Evidence for T-Cell Immunosenescence from Previous Studies on A-Bomb Survivors

Age-associated changes in T-cell populations are primarily characterized as (1) decreases in cell numbers and functions such as proliferative responses to T-cell receptor (TCR) stimulation and T-helper function in naïve T-cell populations, (2) increases in memory T-cell populations, (3) decreased TCR repertoire diversity along with a reduced antigen recognition, and (4) frequent emergence of an oligoclonally expanded population of memory T cells (Fig. 1, left panel) (8). Radiation effects on the human immune system that are in accordance with the age-associated changes in T-cell populations are also depicted in Fig. 1 (right panel). Among A-bomb survivors, we have observed radiation-associated alterations such as (1) decreases in naïve CD4 and CD8 T cells (6, 9–11), (2) increases in memory CD4 and CD8 T cells (6), and (3) decreased repertoire diversity in memory CD4 T cells (11). We also observed (4) clonal chromosome aberrations of memory T-cell origin in heavily exposed survivors, suggesting clonal expansion of memory T cells (12). Thus the effects of radiation on T-cell immunity mostly resemble the effects of aging on the immune system.

Although other studies have reported that clonal expansion of a subset of memory T cells, CD28⁻ or CD57⁺, frequently occurred in older unirradiated

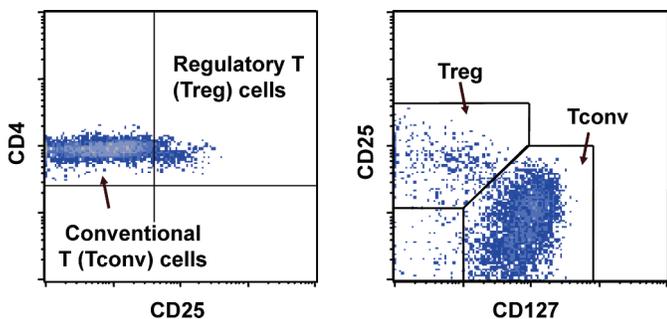


FIG. 2. Flow cytometry patterns of regulatory T (Treg) and conventional T (Tconv) cells that were discriminated with CD25 expression levels alone (left panel) or with both CD25 and CD127 levels (right panel) in the peripheral blood CD4 T-cell fractions from a typical A-bomb survivor.

individuals (8, 13), no significant radiation-associated change in percentages of such cells in either CD4 (14) or CD8 (6) T-cell subsets was observed among A-bomb survivors. To further evaluate the maintenance of T-cell memory in A-bomb survivors, we recently analyzed memory CD4 T-cell subsets using HSCA-2 (recognizing the low-molecular-mass glycoform of CD43), a monoclonal antibody that we established to classify human memory T cells (15). We found that functionally weak (CD43^{middle}) and anergic (CD43^{low}) memory CD4 T-cell subsets dose-dependently increased among A-bomb survivors, suggesting that attrition of the competent memory T-cell population is related to radiation exposure (16).

Immunosenescence in the T-cell system may be involved in long-lasting impairments in T-cell functions among A-bomb survivors. In fact, our previous observations indicated that previous radiation exposure dose-dependently induced deleterious effects on T-cell functions in A-bomb survivors. *In vitro* T-cell proliferative responses to PHA (17) and alloantigens (18) and the frequency of IL-2-producing T cells (19) were all found to decrease in association with radiation dose. Also noted were dose-dependent decreases in the proliferative responses of A-bomb survivors' T cells that were exposed to superantigen staphylococcal enterotoxins (10). The superantigen responses correlated well with the naïve CD4 T cell percentages, suggesting that the immunosenescence in naïve CD4 T cells that we consistently observed in A-bomb survivors might account in part for their deficits of T-cell proliferative function (10). In addition, the other T-cell functions, which were inversely related to radiation, also tended to decrease in association with aging. Taken together, there is accumulating evidence of T-cell immunosenescence being associated with aging and previous radiation exposure in A-bomb survivors and consequently leading to diminished functions in their adaptive immune systems.

Radiation Effects on Regulatory T Cells (Current Study)

CD4⁺ regulatory T (Treg) cells express CD25 and suppress T-cell activation and function, and their

number increases with age (20). Our previous measurement of Treg, which were identified by their high CD25 expression level, did not find any effect of age or radiation on these cells, probably due to the difficulty in accurately discriminating Treg from conventional T (Tconv) cells (data not shown). Recently, however, Treg cells were shown to be clearly identified by their CD25⁺/CD127⁻ phenotype (21, 22), allowing us to make more accurate measurements. Figure 2 shows representative flow cytometry patterns of Treg cells that were identified by the CD25⁺ phenotype alone and by the CD25⁺/CD127⁻ phenotype in an A-bomb survivor. We could not find a significant effect of age on Treg cells (Table 2). This is probably due to the advanced ages of our study subjects; all subjects were 60 years old or older. As for radiation effects, there was a dose-dependent increase in the percentages of Treg cells in the CD4 T-cell population. The same trend was suggested for the ratio between Treg and Tconv cells. Those results suggest that A-bomb radiation might direct T-cell immunity toward suppressor phenotypes in relation to immunosenescence. It has been demonstrated that Treg cells have a strong influence in suppressing pathological immune responses in autoimmune diseases (23). Clinical studies in A-bomb survivors thus far have found no evidence that supports the idea that there is an increase in autoimmune diseases (24). Therefore, the current observation for Treg cell counts is not contradictory to the absence of an excess prevalence of autoimmune diseases in A-bomb survivors.

Radiation Effects on Naïve and Memory CD4 T-Cell Subsets (Current Study)

Naïve and memory T cells are mature T cells that have not previously experienced antigen encounter and that mediate recall responses to second and subsequent exposure to antigens, respectively. Memory T-cell populations are produced by antigen stimulation of naïve T cells and survive for many years and are classified based on the expression of other surface markers including CD62L into central and effector memory T cells and effector T cells with several differences in their functions, migration capacities and proliferation abilities. Our previous examinations of the peripheral blood lymphocyte populations of A-bomb survivors using CD45RO and CD62L as markers showed decreased percentages of naïve CD4 and CD8 T cells and increased percentages of memory CD8 T-cell subsets in a dose-dependent manner (6). In the present study, we discriminated naïve and memory CD4 T-cell subsets by using CD45RA and CD62L as markers and assessed the percentages of these subsets in the peripheral blood CD4 T-cell population among A-bomb survivors. We observed a dose-dependent decrease in the percentage of CD45RA⁺/CD62L⁺ (naïve, T_n) cells within the CD4 T-cell population, confirming

TABLE 2
Age, Gender and Radiation Effects on the Percentages of T-Cell Subpopulations in the Peripheral Blood CD4 T-Cell Populations among a Total of 1,035 A-Bomb Survivors^a

T-cell subpopulation	Estimated percentage for nonirradiated males at 75 years of age	Percentage change of T-cell subpopulation percentage per unit		
		Age (10 years) ^b	Gender ^c	Dose (Gy) ^d
Regulatory T (Treg) and conventional T (Tconv) cells				
CD25 ⁺ /CD127 ⁻ (Treg)	6.3	1.2 (-1.2, 3.5) ^e <i>P</i> = 0.34	-7.3 (-11.7, -5.3) <i>P</i> = 0.0004	2.7 (0.1, 5.3) <i>P</i> = 0.044
25.9CD25 ⁻ /CD127 ⁺ (Tconv)	89.7	-0.8 (-1.2, -0.5) <i>P</i> = 0.0001	0.9 (0.3, 1.5) <i>P</i> = 0.0046	-0.1 (-0.5, 0.3) <i>P</i> = 0.67
Ratio (Treg/Tconv)	7.0	2.0 (-0.5, 4.6) <i>P</i> = 0.13	-8.2 (-12.4, -4.0) <i>P</i> = 0.0002	2.7 (-0.1, 5.5) <i>P</i> = 0.052
Naïve and memory				
CD45RA ⁺ /CD62L ⁺ (Tn)	25.9	-18.7 (-23.5, -13.9) <i>P</i> = 0.0001	5.6 (-2.2, 13.8) <i>P</i> = 0.16	-5.6 (-10.9, -0.3) <i>P</i> = 0.040
CD45RA ⁻ /CD62L ⁺ (Tcm)	44.5	0.4 (-1.6, 2.5) <i>P</i> = 0.68	0.3 (-3.0, 3.7) <i>P</i> = 0.85	4.5 (2.3, 6.8) <i>P</i> = 0.0001
CD45RA ⁻ /CD62L ⁻ (Tem)	19.6	17.3 (13.4, 21.2) <i>P</i> = 0.0001	-2.1 (-8.5, 4.3) <i>P</i> = 0.51	2.5 (-1.8, 6.7) <i>P</i> = 0.26
CD45RA ⁺ /CD62L ⁻ (Teff)	0.4	8.6 (-3.0, 20.2) <i>P</i> = 0.15	-22.3 (-42.1, -2.6) <i>P</i> = 0.036	-8.0 (-21.1, 5.0) <i>P</i> = 0.23

Note. Treg, regulatory T cells; Tconv, conventional T cells; Tn, naïve T cells; Tcm, central memory T cells; Tem, effector memory T cells; Teff, effector T cells.

^a Percentages of CD4 T-cell subpopulations for age, gender and dose were obtained by the multiple regression model, using the following formula: $\log(\text{percentage T cells}) = \alpha + \beta_1 \times \text{age} + \beta_2 \times \text{gender} + \beta_3 \times \text{dose}$, where the value showing zero for the percentage of T cells was replaced with 0.01 when the value was transformed into natural log.

^b Effects of age were estimated for 10 years.

^c Gender = 0 for male and = 1 for female.

^d Effects of dose were estimated for 1 Gy.

^e 95% confidence interval.

our previous findings (Table 2). As for memory T-cell subsets, the percentage of CD45RA⁻/CD62L⁺ (central memory, Tcm) cells appeared to increase with radiation dose, whereas there was no significant radiation effect on CD45RA⁻/CD62L⁻ (effector memory, Tem) or CD45RA⁺/CD62L⁻ (effector, Teff) cells (Table 2). Those results clearly indicate that radiation exposure generated a reduction of Tn cell populations but an increase of Tcm cell populations among A-bomb survivors. Because Treg cells are known to suppress differentiation of resting naïve and memory T cells into effector cells (24), we analyzed the associations between the percentages of Treg cells with the CD25⁺/CD127⁻ phenotype and those of Teff cells with the CD45RA⁺/CD62L⁻ phenotype. A multiple regression analysis showed an inverse association between Treg and effector cell percentages ($r = 0.14$, $P = 0.0001$) after adjusting for age, gender and radiation dose. Although it needs to be determined whether the increase in the proportion of Treg cells is actually responsible for a suppression of the effector functions of T cells, elevated Treg cell levels in A-bomb survivors may be partly involved in the diminished T-cell responsiveness that we have observed among the survivors.

T-Cell Immunosenescence and Inflammation

A link between alterations in T-cell immunity and elevated inflammation among A-bomb survivors is suggested (24). We found that plasma levels of inflammatory cytokines such as IL-6, TNF- α and IFN- γ increased with radiation dose (26). Kim *et al.* (27)

reported that treatment of T-cell-deficient nude and *Rag1*-knockout mice with poly I:C led to a lethal cytokine storm, whereas similar doses of poly I:C did not kill wild-type mice, which possess abundant T lymphocytes. They also showed that T cells, either Tn or Treg, were sufficient to control this cytokine response by the adoptive transfer of T cells prior to poly I:C treatment of *Rag1*-knockout mice, which resulted in decreased pro-inflammatory cytokine production. Those results suggest that T cells suppress the cytokine storm that occurs during the initial innate immune response. In A-bomb survivors, there were dose-dependent increases in plasma levels of inflammatory cytokines and CRP, and such enhanced inflammatory responses might be caused by alterations in the T-cell system of the survivors. For example, we found that plasma IL-6 and CRP levels were significantly elevated in the survivors with low percentages of peripheral blood CD4 T cells (26). A similar trend was also apparent in the survivors with low percentages of Tn (YK and TH, unpublished observation).

To seek a further link of T-cell immunosenescence to inflammatory responses among A-bomb survivors, in the present study we analyzed associations between percentages of T-cell subsets (that we currently examined) and plasma TNF- α levels. TNF- α concentration has already been measured among a large number of subjects for another study and purpose; 1.50 pg/ml was estimated for nonirradiated males at 75 years of age, and the effects (percentage increases) of age, gender and radiation dose

TABLE 3
Partial Correlation Coefficients between TNF- α Levels and Percentages of T-Cell Subsets in the Peripheral Blood CD4 T-Cell Population

	T-cell subset				
	Treg	Tn	Tcm	Tem	Teff
TNF- α	-0.08 ($P = 0.36$)	-0.20 ($P = 0.014$)	0.04 ($P = 0.67$)	0.23 ($P = 0.005$)	0.12 ($P = 0.15$)

Notes. Partial correlation coefficients adjusted for sex, age and radiation dose were calculated after TNF- α levels and percentages of T-cell subsets were naturally log-transformed. Each P value in parentheses is a significant level of partial correlation coefficient between natural log-transformed percentage of the T-cell subset and the log-transformed of TNF- α level. The value showing zero for the percentage of T cells was replaced with 0.01 when the value was transformed into natural log. Treg, regulatory T cells; Tn, naïve T cells; Tcm, central memory T cells; Tem, effector memory T cells; Teff, effector T cells.

on TNF- α concentration were 15% for 10 years, 15% for females relative to males, and 7% for 1 Gy, respectively (28). In this study, we remeasured the TNF- α concentration with a subset (155 survivors) of the current study subjects, whose T-cell data are available in Table 2, and we used these data (1.14 pg/ml being estimated for nonirradiated males at 75 years of age) for the calculation of partial correlation coefficients in Table 3. Plasma TNF- α levels were inversely associated with the percentages of Tn but not Treg cells, suggesting an involvement of Tn but not Treg cells in suppression of innate inflammatory responses manifested by increased TNF- α levels (Table 3). This may explain why we observed long-lasting inflammatory responses despite an increase in the relative number of immunosuppressive Treg cells in A-bomb survivors. It was also noted that plasma TNF- α levels were positively associated with the percentage of Tem cells (Table 3). There was a negative correlation between percentages of Tn and Tem cells ($r = -0.71$, $P = 0.0001$) but not between those of Treg and Tem cells ($r = 0.05$, $P = 0.13$). Thus T-cell immunosenescence manifested by a reduction in the relative number of Tn cells might be linked to the development and/or expansion of Tem cells, involving enhanced inflammatory responses.

Implications of T-Cell Immunosenescence in Disease Development among A-Bomb Survivors

We found previously that the CD4 T-cell percentages were significantly lower in survivors with a history of myocardial infarction (MI) than in survivors with no such history (28). We also noted that the T cells of survivors with a history of MI tended to be poor responders to several superantigens of *S. aureus* toxins and that these same individuals had proportionally fewer naïve CD4 T cells than survivors with no MI history (10). As suggested for a link between alterations in T-cell immunity and inflammation among A-bomb survivors, both IL-6 and CRP levels were significantly higher in survivors with a history of MI than in those without such a history (26). We thus believe that the T-cell immunosenescence associated with inflammatory reactions will prove to be a cause of increased risk of cardiovascular disease among A-bomb survivors, in

much the same way as seen in other epidemiological studies of unirradiated individuals (29–31). However, because it is still possible that the disease itself or some medication might be responsible for such immunosenescence, prospective studies are needed to provide definitive information on the causal link between T-cell immunosenescence and inflammatory diseases.

Noncancer diseases frequently observed in A-bomb survivors include circulatory, respiratory and digestive diseases, especially cardiovascular disease, pneumonia and liver disease (1, 2). One possible explanation involves the failure of aging immune systems to control microbial infections, since infections might lead to chronic inflammation and hence to increased susceptibility to such noncancer diseases in heavily exposed survivors, resulting from unregulated and hence long-lasting inflammatory responses. In A-bomb survivors, however, no significant long-term effects of radiation on the antimicrobial functions of blood monocytes or granulocytes (e.g., phagocytosis, *in vitro* migration, etc.) have been noted (32). There is a growing interest in the role of pathogen recognition molecules, such as Toll-like receptors (TLRs), in the pathogenesis of chronic inflammatory diseases: TLRs play a key role in the host defense against exposure to microbial pathogens and also in the development and progression of atherosclerotic lesions (33, 34). It may be important and possible to conduct an integrated and systematic examination of the A-bomb survivors' innate immune systems in the future, including the analyses of TLR-mediated signaling as well as interaction of the signaling with T-cell immunity.

Conclusions and Perspectives

In summary, A-bomb radiation may have induced T-cell immunosenescence, resulting in attenuation of T-cell-mediated immunity. Such decrements in the T-cell system may cause chronic inflammation and in turn may be partly responsible for cardiovascular disease and other aging-associated diseases of importance. Although there is accumulating evidence of T-cell immunosenescence among A-bomb survivors, how ionizing radiation causes T-cell immunosenescence and how radiation-

induced T-cell immunosenescence interacts with ordinary aging remain to be explained. Mechanistic approaches using appropriate animal models will be necessary to depict a complete picture of radiobiological involvements in host immunological aging. Longitudinal analyses of the changes in the various immunological parameters may provide a suitable vehicle for a better understanding of the interaction between radiation-related and aging-associated immunological changes and for exploring causal relationships between these immunological changes and various noncancer diseases in A-bomb survivors.

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