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Long-Term Effects of Radiation Exposure and Metabolic Status on Telomere Length of Peripheral Blood T Cells in Atomic Bomb Survivors

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In a series of studies of atomic bomb survivors, radiation-dose-dependent alterations in peripheral T-cell populations have been reported. For example, reduced size in naïve T-cell pools and impaired proliferation ability of T cells were observed. Because these alterations are also generally observed with human aging, we hypothesized that radiation exposure may accelerate the aging process of the T-cell immune system. To further test this hypothesis, we conducted cross-sectional analyses of telomere length, a hallmark of cellular aging, of naïve and memory CD4 T cells and total CD8 T cells in the peripheral blood of 620 atomic bomb survivors as it relates to age and radiation dose, using fluorescence *in situ* hybridization with flow cytometry. Since telomere shortening has been recently demonstrated in obesity-related metabolic abnormalities and diseases, the modifying effects of metabolic status were also examined. Our results indicated nonlinear relationships between T-cell telomere length and prior radiation exposure, i.e., longer telomeres with lower dose exposure and a decreasing trend of telomere length with individuals exposed to doses higher than 0.5 Gy. There were associations between shorter T-cell telomeres and higher hemoglobin Alc levels or fatty liver development. In naïve and memory CD4 T cells, radiation dose and high-density lipoprotein (HDL) cholesterol were found to positively interact with telomere length, suggesting that the decreasing trend of telomere length from a higher radiation dose was less conspicuous in individuals with a higher HDL cholesterol. It is therefore likely that radiation exposure perturbs T-cell homeostasis involving telomere length maintenance by multiple biological mechanisms, depending on dose, and that long-term-radiation-induced effects on the maintenance of T-cell telomeres may be modified by the subsequent metabolic conditions of individuals.

INTRODUCTION

Homeostasis of the T-cell immune system plays a key role in human health as well as in the development of various diseases (*1, 2*), and this system is highly vulnerable to the effects of radiation exposure (*3*). Immunological studies in atomic bomb (A-bomb) survivors have indicated several radiation-dose-dependent alterations in peripheral T-cell populations: reduced pool sizes of naïve CD4 T and naïve CD8 T cells; increased pool sizes of memory T cells; impaired abilities of T cells to proliferate in response to *in vitro* antigen stimulation; and decreased diversity of T-cell receptor repertoire in memory CD4 T cells (*3*). Because such T-cell alterations are also typically observed with human aging (*4*), we hypothesized that radiation accelerates the natural aging process of T-cell immunity by disrupting the maintenance of T-cell homeostasis.

The reduced naïve T-cell pool and impaired T-cell proliferation ability observed in A-bomb survivors have raised the question of whether radiation accelerates the shortening of T-cell telomeres. Telomeres are nucleoprotein structures with tandem repeats of 5′-TTAGGG-3′ DNA sequences at the ends of chromosomes; they prevent chromosome end fusions and ensure proper chromosome replication (*5, 6*). The telomere length in normal human somatic cells shortens with each cell division, mainly due to an incomplete replication of telomere ends. If telomere shortening continues to a critical length, it induces cell-cycle arrest, cellular senescence and apoptotic cell death (*5*). Because telomere length thus reflects cellular replicative history and restricts the ability to proliferate, it represents a major biomarker of T-cell aging.

To date, most studies focusing on radiation exposure and telomere length have been conducted in cultured cells or in animal models shortly after irradiation (*7*). Although long-term effects of radiation on telomere length may have profound implications for human health, the limited reported studies using human peripheral blood leukocytes or lymphocytes have had mixed results, i.e., either shortening, elongation or no significant changes (*8–10*). Such results might stem from different types of ionizing radiation, varying radiation doses/dose rates and the passage

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TABLE 1
Study Participants (N = 620)

Basic characteristics	
Age at time of bombing[a]	15.9 (2.1–29.5)
Age at the time of examination[a]	77.2 (62.7–90.0)
Gender[b]	
Male	203 (32.7)
Female	417 (67.3)
Radiation dose (Gy)[a]	0.102 (0.000–1.736)
Alcohol (grams/day)[a]	0 (0–40)
Alcohol[b]	
0	411 (66.3)
0–20	154 (24.8)
20–40	30 (4.8)
40–60	17 (2.7)
>60	8 (1.3)
Smoking (cigarettes/day)[a]	0 (0–15)
Smoking[b]	
0	556 (89.7)
0–20	61 (9.8)
>20	3 (0.5)

Metabolic indicators	
BMI (kg/m^2)[a]	22.9 (17.4–28.4)
BMI[b]	
≤19.5	84 (14.8)
19.5–21.2	98 (17.3)
21.2–22.9	102 (18.0)
22.9–25.0	143 (25.2)
>25.0	141 (24.8)
Total cholesterol (mg/dl)[a]	209.0 (156.0–266.0)
HDL cholesterol (mg/dl)[a]	55.3 (35.8–83.1)
LDL cholesterol (mg/dl)[a]	117.5 (75.7–168.7)
HbA1c (%)[a]	5.8 (5.1–7.9)
CRP (mg/dl)[a]	0.08 (0.01–0.71)
Ischemic heart disease case[b]	73 (11.8)
Diabetes case[b]	121 (19.5)
Fatty liver case[b]	184 (29.7)
Hypertension case[b]	434 (70.0)

[a] Median range (5–95%).
[b] Number (%).

of time after radiation exposure, as well as from modifying factors such as alcohol consumption, smoking, medical history and obesity (*11*). Furthermore, a recently reported study clearly showed that changes in telomere length with aging occurred at different rates in different cell subsets (T cells, B cells and monocytes), and that proportions of cell subsets within blood cells also underwent significant changes with aging (*12*), suggesting the need to separately analyze telomere length in different cell subsets.

In this study, we evaluated long-term radiation effects on telomere length and tested our primary hypothesis, that telomere shortening in T-cell population aging may be accelerated by radiation. To accomplish this, we examined telomere length of naïve and memory CD4 T cells, total CD8 T cells and granulocytes in peripheral blood from 620 A-bomb survivors in Hiroshima. In addition, we tested a second hypothesis, that long-term radiation effects on telomere length in T cells may be modified by metabolic conditions and diseases of the host. This was based on emerging evidence of telomere erosion with obesity-related metabolic abnormalities and diseases (*6, 13–16*). Also of importance are the reported data of metabolic abnormalities, including higher total serum cholesterol and lower high-density lipoprotein (HDL) cholesterol levels, as well as fatty liver development, which have been observed in association with radiation dose in A-bomb survivors (*17–19*).

MATERIALS AND METHODS

Study Participants

The Adult Health Study (AHS) cohort was established in 1958, and has enrolled 23,000 A-bomb survivors in Hiroshima and Nagasaki who undergo biennial health examinations at the outpatient clinics of the Radiation Effects Research Foundation (RERF) (*20*). Approximately 1,100 Hiroshima participants in the AHS were randomly selected to examine immunological phenotypes (*21, 22*). Of these participants, telomere length was measured for 792 survivors who donated blood samples in 2003–2009. We excluded 171 participants who had been diagnosed with cancer, due to the potential effects of cancer development or therapeutics on telomere length, as well as one participant who had a missing value for smoking, resulting in 620 participants for statistical analyses (Table 1). Radiation dose was the weighted absorbed bone marrow dose (Gy), based on the Dosimetry System 2002 and recent updates (*23–25*).

This study was approved by the RERF Human Investigation Committee, and was conducted according to the principles expressed in the Declaration of Helsinki. All participants gave written informed consent before each examination.

Measurement of Specific Telomere Fluorescence by Flow-FISH

Peripheral blood mononuclear cell (PBMC) fractions were collected from the upper layer of the Ficoll-Hypaque gradient sedimentation of fresh heparinized blood samples. Granulocyte fractions were obtained by hemolysis of buffy coat blood cells collected from the lower layer of the blood sedimentation. Approximately 10 million PBMCs were stained with fluorescein isothiocyanate (FITC)-labeled anti-CD4 antibody (BD Biosciences, San Jose, CA), phycoerythrin (PE)-labeled anti-CD45RA antibody (Beckman Coulter® Inc., Fullerton, CA) and PE-Cy5-labeled anti-CD8 (BD Biosciences). Naïve ($CD4^+CD45RA^+$) and memory ($CD4^+CD45RA^-$) CD4 T cells and total CD8 T cells

($CD8^{bright}$) were sorted using a FACSVantage™ (BD Biosciences). The telomere lengths in these T-cell fractions and granulocytes were measured using fluorescence *in situ* hybridization (FISH) as previously described by Rufer *et al* (*26*), using 1×10^5 cells of each of the cell fractions. In brief, this procedure involved DNA denaturation at 85°C for 10 min and hybridization at 20°C overnight in 70% formamide, 20 m*M* Tris-HCl and 0.1% bovine serum albumin solution with FITC-labeled telomere-specific $(CCCTAA)_3$ or telomere–unspecific (CCC ATA ACT AAA CAC: the alphoid sequences of the X chromosome) peptide nucleic acid (PNA) probes (Sawady Technology Inc., Tokyo, Japan). After treatment of the cells with RNase, the interphase cell fraction was determined by measuring the fluorescence of 7-aminoactinomycin-D that binds to cellular DNA. FITC fluorescent intensity and its distribution in the interphase cells were measured using a FACScan™ (BD Biosciences). Specific telomere fluorescence was determined by subtracting the mean fluorescent intensity with telomere-unspecific probes from that with telomere-specific probes. In addition, we confirmed the reproducibility of the flow-FISH method and found highly correlated results between this fluorescence-based measurement and a Southern blot method in our previous study (*27*).

Lifestyle Factors and Clinical Information

Lifestyle and clinical information were obtained as previously described (*22*). Information on alcohol consumption and smoking at the time of telomere measurement was obtained from questionnaires at the AHS health examinations in 2003–2009. Clinical information such as body mass index (BMI), total serum cholesterol, HDL cholesterol, low-density lipoprotein (LDL) cholesterol, hemoglobin A1c (HbA1c), C-reactive protein (CRP) levels and diseases was also obtained at these health examinations. The following conditions (according to ICD-10 codes) were classified and included in the statistical analyses: cancer (C00–C97, D00–D09), ischemic heart disease (I20–I25), type-2 diabetes (E11–E14, based on blood HbA1c and glucose levels as well as treatment information), fatty liver (K70.0 and K76.0, based on abdominal ultrasonography findings) and hypertension (I10–I15).

Statistical Data Analysis

The association between each telomere length measurement and radiation exposure was evaluated with multiple linear regression, taking the natural log-transformation of telomere length. Considering the highly right-skewed distribution of the radiation dose in A-bomb survivors, we evaluated the effects of high-dose points by comparing the regression results of natural log-transformed telomere length and original-scale radiation dose to the results of methods such as super smoothers in nonparametric smoothing, in which it was known that the results of the analysis would be robust to the effect of influential data points, and to the results of analyses based on log-transformed telomere length and log-transformed radiation dose. We took the logarithm of the radiation dose by adding a small number (added 0.005 Gy) to avoid taking log of zero. The analysis was adjusted for continuous variables of participant age at examination, alcohol consumption and quantity of smoking; an indicator variable of gender; and metabolic-condition-related continuous variables (BMI, HbA1c, total cholesterol, HDL cholesterol, LDL cholesterol and CRP) and indicator variables of diseases (ischemic heart disease, type-2 diabetes, fatty liver and hypertension) possibly related to telomere length. The scatter plots of each telomere length measurement for radiation dose as well as age showed high variability among individuals, and we used both linear terms as well as nonlinear terms of age and radiation dose to detect complex changes in telomere length as it relates to aging and radiation exposure in the model selection. The starting model consisted of the main effects of the linear terms of age and dose, the quadratic terms of age and dose, the cubic term of dose and all of the candidate covariates. The stepwise variable selection based on the Akaike Information Criterion (AIC) was then applied to evaluate the association with telomere lengths using the ''step'' function of R statistical software (http://cran.r-project.org). Because we were investigating the existence of associations between the telomere length of each subset and the metabolic status of the participants, we selected the models with the minimum AIC of each end point and evaluated the association of radiation exposure with telomere lengths with adjustment of selected sets of variables. When none of the radiation terms was included in the model, association between telomere length and a linear term of radiation was shown. The model fits of polynomial functions of radiation dose for each end point were also compared by the AIC with that of the dose-response model commonly considered due to radiation-induced effects, such as sterilizing (or cell killing): $(\beta_1 d + \beta_2 d^2)\, e^{\gamma d}$, where d is the radiation dose, β_1 and β_2 are regression coefficients of linear and quadratic terms of radiation dose, respectively, and γ is the additional regression coefficient of the sterilizing effect of $\gamma < 0$. Additionally, mean telomere lengths were compared by radiation dose category (0–0.005 Gy, 0.005–0.5 Gy, 0.5–1 Gy and $\geq$1 Gy), adjusting for selected covariates in the model selection. The final parametric model was decided by the AIC and by comparing the shape of the parametric function of selected radiation terms to that of nonparametric estimates and also to the smoothing curve of residual plots of the final model without radiation terms. Then, super smoother was mainly used as a method of nonparametric smoothing to lessen the effect of influential points and outliers.

In addition, recent evidence from mouse studies suggests direct interactions between individual metabolic status and biological responses to radiation exposure (*28, 29*). Therefore, the difference in the association between radiation dose and telomere length with different values of metabolic conditions or diseases was investigated by testing the two-way multiplicative interaction of radiation terms and each metabolic indicator by adding those variables to the selected best models of each subset in the main effect investigation mentioned above. We also considered dividing the radiation dose range because we may not be able to ignore the possibility that different biological mechanisms may underlie some responses to high-dose radiation; i.e., the association was investigated separately for individuals with radiation exposure $<$0.5 and $\geq$0.5 Gy, at which lymphocyte apoptosis is generally induced. All of the tests were two-sided, and analyses were conducted using R software version 3.2.1.

RESULTS

Telomere Length and Aging

The basic characteristics of the study participants, a subset of the A-bomb survivor cohort, are shown in Table 1, and a representative flow cytometry pattern is shown in Fig. 1. We conducted multiple regression analyses using age at examination, gender, radiation dose, alcohol consumption and smoking along with metabolic conditions and diseases, to find potential factors that might influence telomere length in peripheral blood T-cell populations and granulocytes. To detect complex changes in telomere length related to aging and radiation exposure, we used not only linear terms but also nonlinear terms for age and radiation dose in the statistical models. The results showed that the coefficients of telomere length were negative for the linear term of age while positive for the quadratic term of age (age^2) in all of the cell populations examined (Table 2). These results suggest that telomere length decreased with age, and did so at a slower rate at around 80 years of age (Fig. 2).

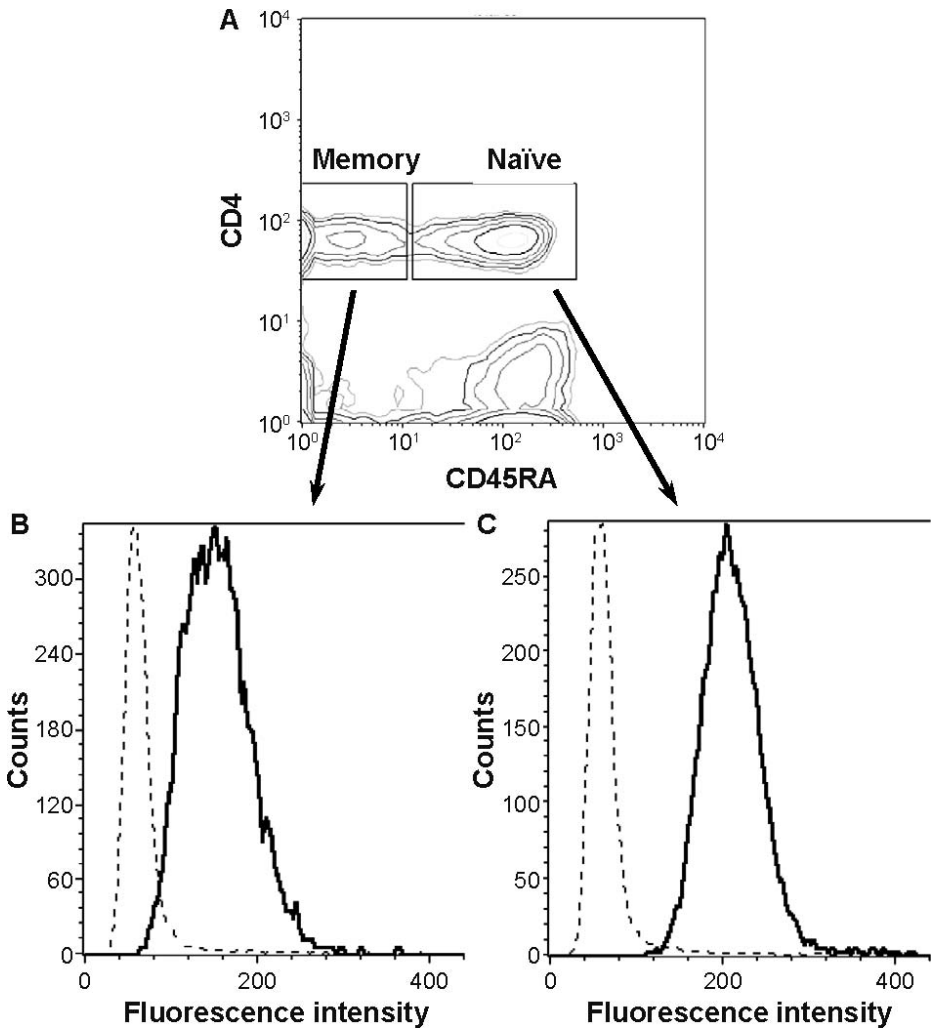

FIG. 1. A representative flow cytometry pattern in flow-FISH. Panel A: Typical flow cytometry pattern of naïve ($CD45RA^+$) CD4 T and memory ($CD45RA^-$) CD4 T cells in an A-bomb survivor. Panels B and C: Fluorescence intensity in each T-cell subset assessed by flow-FISH with telomere-unspecific (dashed lines) and telomere-specific probes (solid lines) in the same survivor.

TABLE 2
Regression Analyses of Telomere Length in Peripheral Blood Cell Populations

Selected variables for naïve CD4 T cells	Coefficient	*P* value	Selected variables for memory CD4 T cells	Coefficient	*P* value
Intercept	5.13	<0.001	Intercept	5.06	<0.001
Age	–0.15	<0.001	Age	–0.17	<0.001
Age^2	0.04	0.019	Age^2	0.04	0.034
Radiation dose		NS[a]	Radiation dose	0.179	<0.001
Radiation $dose^2$	0.132	0.002	Radiation $dose^2$		NS[a]
Radiation $dose^3$	–0.046	0.002	Radiation $dose^3$	–0.021	0.002
HbA1c	–0.04	0.028	HbA1c	–0.04	0.036
Fatty liver	–0.1	0.004	Fatty liver	–0.09	0.022
Selected variables for CD8 T cells	**Coefficient**	***P* value**	**Selected variables for granulocytes**	**Coefficient**	***P* value**
Intercept	4.87	<0.001	Intercept	4.97	<0.001
Age	–0.18	<0.001	Age	–0.09	0.002
Age^2	0.03	0.15	Age^2	0.04	0.036
Radiation dose	0.164	0.004	Radiation dose	0.1	0.083
Radiation $dose^2$		NS[a]	Radiation $dose^2$		NS[a]
Radiation $dose^3$	–0.02	0.007	Radiation $dose^3$	–0.04	0.1
HbA1c	–0.04	0.062	Ischemic heart disease	–0.07	0.14
Fatty liver	–0.11	0.02	HbA1c	–0.04	0.022

[a] Not significant.

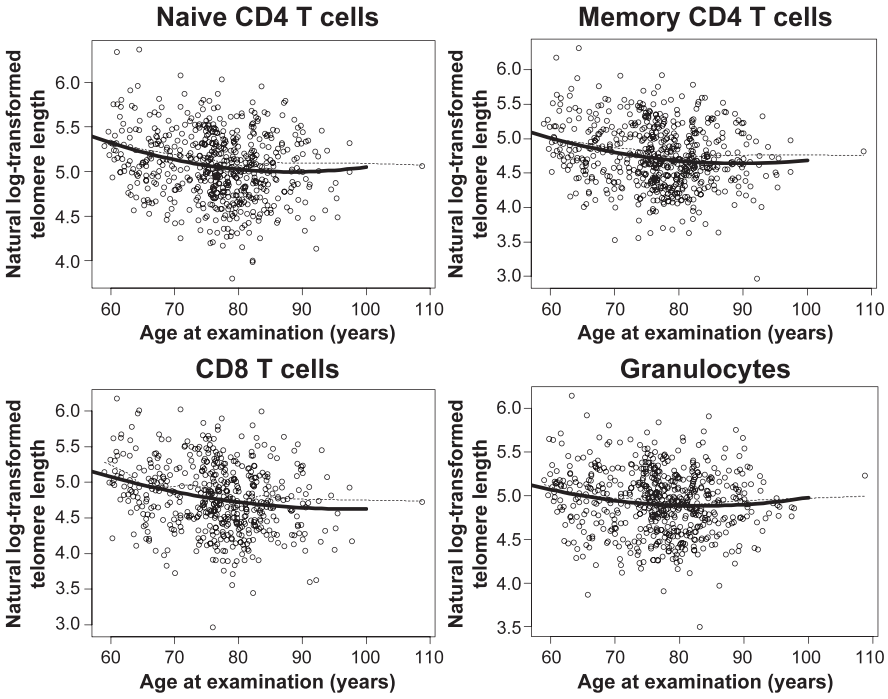

FIG. 2. Telomere length with age in peripheral T-cell populations and granulocytes. Each dot represents a measured value of telomere length. Specific telomere fluorescence was determined by subtracting the mean fluorescent intensity with telomere-unspecific probes from that with telomere-specific probes, and was then natural log-transformed. The solid curves in each panel represent predicted values obtained from parametric regression models. The thin curves represent nonparametric smoothers of the residuals of the multivariable regression model as a function of age. These overlapping curves are estimated for individuals who were not exposed to A-bomb radiation and who had the median HbA1c value and no fatty liver.

Telomere Length and Radiation Exposure

Analysis of telomere length and radiation exposure indicated significant associations between telomere length and dose in naïve and memory CD4 and total CD8 T-cell populations, i.e., positive coefficients of telomere length for linear terms or a quadratic term of radiation dose (radiation dose^2) and negative coefficients for cubic terms of radiation dose (radiation dose^3; Table 2 and Fig. 3). Similar results were obtained in linear-quadratic models and nonlinear models, but the AIC of the final models showed a better fit than the other models (data not shown). In addition, comparison of the mean telomere length of radiation dose categories showed that there was a significant increase in the 0.5–1 Gy category (the mean telomere lengths in arbitrary units, a nonlogarithmic scale: 187.0, 145.7, 149.0) compared to the lowest dose category (0–0.005 Gy, the mean telomere lengths: 170.0, 122.3, 129.6) in naïve CD4, memory CD4 and CD8 T-cell populations ($P = 0.04$, $P = 0.002$, $P = 0.03$), respectively; there was a slight but statistically-insignificant decrease in the higher dose category ($\geq$1 Gy, the mean telomere lengths: 183.2, 138.0, 144.3) compared to the 0.5–1 Gy category. Telomere lengths were not significantly different between the 0–0.005 and $\geq$1 Gy dose categories (data not shown). We further examined the relationship between telomere length and log-transformed radiation dose since, by taking the log of the radiation dose, the study participants were relatively equally distributed in terms of radiation dose, and it was possible that such log transformation of the dose might weaken the influence of the small number of higher dose individuals in the regression analysis. The nonparametric smoothing curves using super smoother of residuals obtained in regression models without radiation terms showed a steeper decreasing trend in the telomere length of three T-cell populations when the dose was greater than approximately 0.5–1 Gy (see the thin curves shown in Fig. 3). On the other hand, those decreasing trends were not statistically captured in the parametric modeling using a log-radiation dose. Based on the model selection, the parametric shape of regression results between the telomere length and log-radiation dose was as follows: a linear function of log dose in memory CD4 T cells ($P = 0.02$) with a positive coefficient of 0.02, and a quadratic term of log dose in CD8 T cells ($P = 0.07$) with a negative coefficient of -0.003. However, when we focused on a narrower domain of radiation dose by dividing the dose range into two; i.e., when individuals with $<$0.5 Gy and those with $\geq$0.5 Gy exposure were separately analyzed, inverse associations between telomere length and radiation dose were observed at doses of $\geq$0.5 Gy, using both original-scale and log-transformed radiation doses (Tables 3 and 4). These data suggest that there were tendencies for radiation-associated telomere shortening at doses higher than approximately 0.5

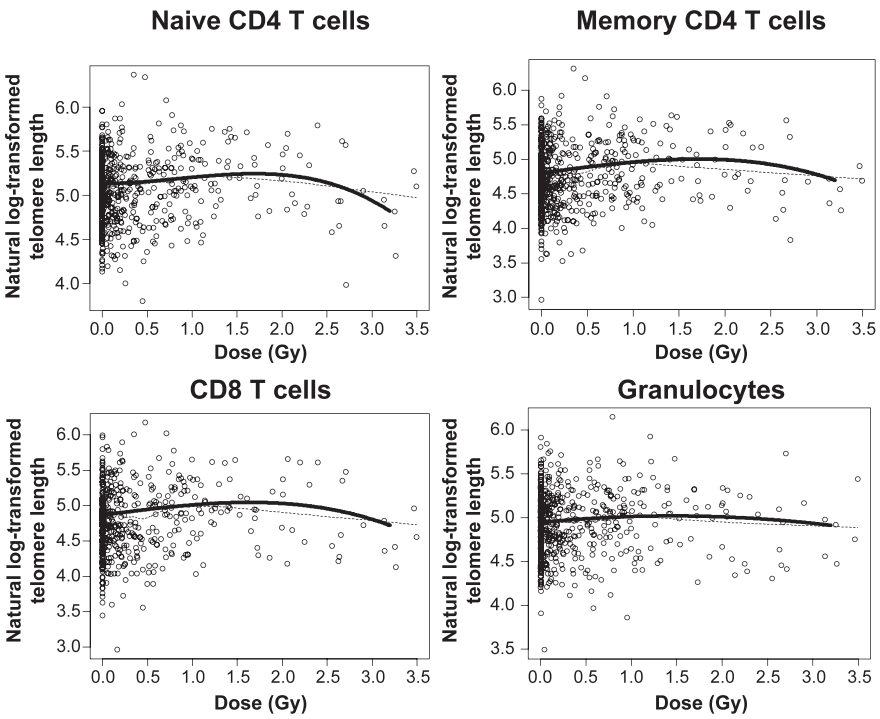

FIG. 3. Telomere length with radiation dose in peripheral T-cell populations and granulocytes. Each dot represents a natural log-transformed measured value of telomere length. Solid curves represent predicted values obtained from parametric regression models. The thin curves represent nonparametric smoothers of each scatter plot between radiation and residuals of multivariable parametric regression. These overlapping curves are estimated for individuals who are 70 years old, with median HbA1c value and no fatty liver.

Gy, whereas telomeres could be elongated with exposure to lower doses of radiation. We observed no significant radiation effects on telomere length in granulocytes.

Telomere Length and Metabolic Status

Metabolic parameters (BMI, total, HDL and LDL cholesterols, HbA1c and CRP) and diseases (ischemic heart disease, type-2 diabetes, fatty liver and hypertension) were included in the regression models as candidate factors related to telomere length. Most of these parameters were not significantly associated with telomere length in T-cell populations. However, we found significant inverse associations between telomere length and HbA1c or fatty liver development in both naïve and memory CD4 and total CD8 T-cell populations (Table 2). In addition, although we employed BMI imputation for individuals who had missing BMI data in the presented analyses, analyses excluding such individuals yielded similar results (data not shown).

Finally, we investigated the significance of interaction terms between radiation dose and each metabolic parameter or disease with telomere length in T-cell populations. The interaction term of CRP and linear radiation dose was found to be significantly associated with telomere length in naïve and memory CD4 T-cell populations ($P = 0.03$ and 0.036, Table 5) with negative coefficients in the nonlinear relationships between radiation and telomere length. The interaction terms were not statistically associated with telomere length when individuals with <0.5 and ≥ 0.5 Gy were separately analyzed. Furthermore, the interaction term of cubic radiation dose and HDL cholesterol was selected in the naïve and memory CD4 T-cell populations in the model selection ($P = 0.07$ and 0.06, respectively). When we looked only at those individuals exposed to ≥ 0.5 Gy radiation, the interaction term was statistically significant in naïve and memory CD4 T-cell populations ($P = 0.008$ and 0.029, respectively; Table 5), indicating that individuals who had a higher HDL cholesterol had a weaker decreasing trend of telomere length with radiation doses. Similar results were obtained for the interactions of radiation dose and CRP or HDL cholesterol using the log-transformed radiation dose (data not shown). Because we individually selected models to evaluate associations between radiation dose and telomere length, including interaction terms using the AIC, correction for multiple testing was not considered.

DISCUSSION

In this cross-sectional study, we investigated effects of prior radiation exposure, which occurred over 60 years ago, on telomere length in peripheral T-cell populations among A-bomb survivors. We first observed shorter telomeres with advanced ages in all of the cell populations of the study participants up to approximately 80 years of age. This finding is in accordance with a recent notion regarding age-related changes in telomere length in human PBMCs or

TABLE 3
Regression Analyses of Telomere Length for Two Separate Dose Ranges (Original-Scale Radiation Dose)

0–0.5 Gy radiation			≥0.5 Gy radiation		
Selected variables for naïve CD4 T cells	Coefficient	*P* value	Selected variables for naïve CD4 T cells	Coefficient	*P* value
Intercept	5.15	<0.001	Intercept	5.08	<0.001
Age	–0.19	<0.001	Age	–0.1	0.018
Age^2	0.06	0.005	Radiation dose	0.31	0.086
Radiation dose^2	–0.66	0.098	Radiation dose^2	–0.11	0.035
HbA1c	–0.05	0.038	Smoking	–0.01	0.08
CRP	0.06	0.082	Fatty liver	–0.12	0.045
Fatty liver	–0.11	0.008			
Selected variables for memory CD4 T cells	Coefficient	*P* value	Selected variables for memory CD4 T cells	Coefficient	*P* value
Intercept	4.83	<0.001	Intercept	5.02	<0.001
Age	–0.24	<0.001	Age	–0.1	0.026
Radiation dose	–0.14	0.4	Radiation dose^2	–0.03	0.06
HbA1c	–0.05	0.06	Smoking	–0.01	0.099
CRP	0.08	0.06	Fatty liver	–0.11	0.12
Fatty liver	–0.11	0.02			
Selected variables for CD8 T cells	Coefficient	*P* value	Selected variables for CD8 T cells	Coefficient	*P* value
Intercept	4.9	<0.001	Intercept	4.85	<0.001
Age	–0.24	<0.001	Age	–0.14	0.01
Age^2	0.06	0.066	Radiation dose	0.33	0.16
Radiation dose	0.09	0.7	Radiation dose^2	–0.12	0.062
HbA1c	–0.06	0.037	Smoking	–0.01	0.099
CRP	0.08	0.1	LDL cholesterol	–0.003	0.062
Fatty liver	–0.12	0.02	Fatty liver	–0.11	0.15

leukocytes (*30*). We then found that telomere lengths in T-cell populations were longer with increasing radiation dose at lower doses and became shorter after exposure to higher doses of A-bomb radiation (Table 2 and Fig. 3). Although telomere lengths were not significantly different between the 0–0.005 and ≥1 Gy dose categories, radiation-associated telomere shortening was observed in individuals exposed to ≥0.5 Gy (Tables 3 and 4). Radiation-associated

TABLE 4
Regression Analyses of Telomere Length for Two Separate Dose Ranges (Log-Transformed Radiation Dose)

0–0.5 Gy radiation			≥0.5 Gy radiation		
Selected variables for naïve CD4 T cells	Coefficient	*P* value	Selected variables for naïve CD4 T cells	Coefficient	*P* value
Intercept	5.11	<0.001	Intercept	5.3	<0.001
Age	–0.18	<0.001	Age	–0.11	0.01
Age^2	0.05	0.006	Radiation dose^2	–0.189	0.028
Radiation dose	–0.008	0.5	Smoking	–0.01	0.066
HbA1c	–0.05	0.038	Fatty liver	–0.12	0.045
CRP	0.06	0.089			
Fatty liver	–0.11	0.007			
Selected variables for memory CD4 T cells	Coefficient	*P* value	Selected variables for memory CD4 T cells	Coefficient	*P* value
Intercept	4.81	<0.001	Intercept	5.02	<0.001
Age	–0.23	<0.001	Age	–0.1	0.038
Age^2	0.07	0.003	Radiation dose^2	–0.2	0.043
Radiation dose	–0.003	0.8	Smoking	–0.01	0.1
HbA1c	–0.05	0.062	Fatty liver	–0.11	0.12
CRP	0.08	0.061			
Fatty liver	–0.11	0.021			
Selected variables for CD8 T cells	Coefficient	*P* value	Selected variables for CD8 T cells	Coefficient	*P* value
Intercept	4.9	<0.001	Intercept	5.08	<0.001
Age	–0.24	<0.001	Age	–0.15	0.006
Age^2	0.06	0.024	Radiation dose^2	–0.26	0.015
Radiation dose	0.004	0.8	Smoking	–0.01	0.084
HbA1c	–0.06	0.038	LDL cholesterol	–0.003	0.064
CRP	0.08	0.095	Fatty liver	–0.11	0.15
Fatty liver	−0.13	0.022			

TABLE 5
Associations of Interaction Terms between Radiation Dose and Metabolic Indicators with Telomere Length

Whole dose range			≥0.5 Gy radiation		
Selected variables for naïve CD4 T cells	Coefficient	*P* value	Selected variables for naïve CD4 T cells	Coefficient	*P* value
Intercept	5.11	<0.001	Intercept	5.29	<0.001
Age	–0.15	<0.001	Age	–0.11	0.006
Age^2	0.04	0.023	Radiation dose^2	–0.02	0.14
Radiation dose	0.16	<0.001	Smoking	–0.01	0.041
Radiation dose^3	–0.02	0.002	HDL cholesterol	–0.005	0.058
CRP	0.07	0.045	Fatty liver	–0.11	0.071
HbA1c	–0.04	0.016	Radiation dose^2 × HDL cholesterol[a]	0.002	0.008
Fatty liver	–0.1	0.003			
Radiation dose × CRP[a]	–0.16	0.03			
Selected variables for memory CD4 T cells	Coefficient	*P* value	Selected variables for memory CD4 T cells	Coefficient	*P* value
Intercept	4.79	<0.001	Intercept	4.97	<0.001
Age	–0.17	<0.001	Age	–0.09	0.041
Age^2	0.04	0.034	Radiation dose^2	–0.02	0.13
Radiation dose	0.22	<0.001	Smoking	–0.01	0.12
Radiation dose^3	–0.02	0.001	HDL cholesterol	–0.004	0.2
HbA1c	–0.05	0.02	Radiation dose^2 × HDL cholesterol[a]	0.002	0.029
Fatty liver	–0.1	0.016			
Radiation dose × CRP[a]	–0.17	0.036			

[a] Interaction terms.

telomere length changes found in the T-cell populations but not in granulocytes may be attributed to indirect but not direct effects of radiation on the hematopoietic system. For example, such telomere length changes may be related to peripheral T-cell responses to cytokine/costimulatory signaling from other somatic cells that might have been functionally altered by radiation exposure. Only in the case of high-dose irradiation are the results supportive of our hypothesis that T-cell telomere shortening with aging may be accelerated by radiation.

A previously reported study using peripheral lymphocytes of Chernobyl cleanup workers showed telomere shortening with radiation exposure (*8*), while another study using peripheral leukocytes of a different cleanup worker population suggested that longer telomeres were associated with radiation exposure as well as with cancer development (*10*). However, when we included 171 individuals diagnosed with cancer in statistical models (791 individuals in total), we still obtained the same results with more statistical significance, i.e., shorter telomeres in T-cell populations at higher dose exposure (data not shown). Although the small number of high-dose-irradiated individuals is a limitation of the current study, a very recent study of leukocyte telomeres also suggested a trend of reduced telomere length with radiation dose in a different subset of A-bomb survivors (*31*), in which more individuals exposed to high-dose radiation were selected than in this study. Thus, differences among studies could be partly explained by different distributions of radiation dose, in addition to different methods of telomere length measurement. Our observations of a nonlinear relationship between T-cell telomere length and radiation exposure (Fig. 3) suggest the possibility that different biological mechanisms may underlie telomere length changes, depending on the radiation dose or the dose rate. High-dose radiation typically induces cell death of lymphocytes, and the susceptibility of lymphocytes to ionizing radiation is higher than that of neutrophils (90–95% granulocytes are neutrophils); 1–2 Gy doses cause lymphocyte loss to 50% of normal within 48 h (*32*). Compensatory cell proliferation after cell death may thus be a major factor for T-cell telomere shortening, which is relevant to our current observations in individuals exposed to higher than approximately 0.5 Gy. On the other hand, in the setting of radiotherapy, it has been reported that peripheral leukocytes with shorter telomeres tended to disappear from the cell population, suggesting that such a cell selection represents a telomere-elongation mechanism (*9*). However, numerous other factors such as telomerase activity after irradiation, telomere maintenance proteins, DNA repair machinery, inflammation (a combination of higher radiation doses and higher CRP levels was suggested to lead to T-cell telomere shortening, as shown in Table 5), oxidative stress and microbial infection, as well as heritable telomere length, are also likely to shape the long-term outcome of telomere length after irradiation (*11, 33–35*). It is further known that the influence of telomeres on diseases depends on the context, such as specific genetic defects (*36*). The use of causal network analysis that takes into account as many relevant factors as practicable, as well as interactions among those factors, may help clarify the causal relationships among radiation exposure, telomere dynamics and disease risk in longitudinal clinical studies.

The inverse associations of T-cell telomere length with HbA1c (Table 2) were consistent with previous findings using leukocytes or T cells (*12, 13, 15, 16*), whereas the associations between shorter telomeres in peripheral T-cell

populations and fatty liver development (*37*) have been newly noted in this study. Therefore, these results support our second hypothesis that long-term radiation effects on telomere length in T cells may be modified by metabolic conditions and diseases of individuals. Generally, obesity-related metabolic status is believed to contribute to telomere attrition by, e.g., oxidative damage to the telomere DNA region, or by promoting peripheral T-cell proliferation through inflammation and high blood glucose levels (*15*). On the other hand, the opposite direction of action is also possible. Senescent T cells are known to produce the inflammatory cytokines TNF-α and IL-6 (*38*), and a causative role of T cells has been demonstrated in the regulation of body weight, fat mass and insulin resistance (*39, 40*). Indeed, a mouse model study showed that shorter telomeres or telomere dysfunction increased the risks of metabolic abnormalities and diseases (*41*). Noteworthy are increased metabolic abnormalities such as elevated total cholesterol level, triglyceride level, trunk-to-limb fat ratio (an indicator of central obesity) and fatty liver development that are associated with radiation dose among A-bomb survivors (*17–19*). Therefore, a bidirectional relationship may exist between T-cell aging and metabolic abnormality, especially in heavily exposed A-bomb survivors, and such a vicious cycle may contribute to the etiology and pathogenesis of type-2 diabetes and fatty liver disease among A-bomb survivors.

In addition to the interactions with CRP, we found positive interaction effects of radiation dose with HDL cholesterol on telomere length in CD4 T-cell populations, especially at doses higher than 0.5 Gy (Table 5). Higher HDL cholesterol levels are often associated with decreased risks of atherosclerosis and type-2 diabetes. Interestingly, in recently published human population studies it has been reported that a slower rate of leukocyte telomere shortening with aging was associated with higher HDL cholesterol, and the authors of those studies cited the anti-oxidant and anti-inflammatory properties of HDL cholesterol (*14, 42*). Although the effects of HDL cholesterol on telomere length mainly relied on the interaction terms, T-cell telomere shortening with radiation doses might be somewhat suppressed in those who had higher HDL cholesterol. More extensive biomarkers involving metabolic and immunologic statuses are yet to be investigated. Nevertheless, the results raise the possibility that long-term radiation effects on the T-cell immune system may be partly alleviated by controlling metabolic conditions of individuals after radiation exposure. Further studies are needed to investigate the relationships that exist among radiation, T-cell homeostasis and metabolic status at both the organism and intracellular levels.

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