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Metabolic Profile as a Potential Modifier of Long-Term Radiation Effects on Peripheral Lymphocyte Subsets in Atomic Bomb Survivors

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Immune system impairments reflected by the composition and function of circulating lymphocytes are still observed in atomic bomb survivors, and metabolic abnormalities including altered blood triglyceride and cholesterol levels have also been detected in such survivors. Based on closely related features of immune and metabolic profiles of individuals, we investigated the hypothesis that long-term effects of radiation exposure on lymphocyte subsets might be modified by metabolic profiles in 3,113 atomic bomb survivors who participated in health examinations at the Radiation Effect Research Foundation, Hiroshima and Nagasaki, in 2000–2002. The lymphocyte subsets analyzed involved T-, B- and NK-cell subsets, and their percentages in the lymphocyte fraction were assessed using flow cytometry. Health examinations included metabolic indicators, body mass index, serum levels of total cholesterol, high-density lipoprotein cholesterol, C-reactive protein and hemoglobin A1c, as well as diabetes and fatty liver diagnoses. Standard regression analyses indicated that several metabolic indicators of obesity/related disease, particularly high-density lipoprotein cholesterol levels, were positively associated with type-1 helper T- and B-cell percentages but were inversely associated with naïve CD4 T and NK cells. A regression analysis adjusted for high-density lipoprotein cholesterol revealed a radiation dose relationship with increasing NK-cell percentage. Additionally, an interaction effect was suggested between radiation dose and C-reactive protein on B-cell percentage with a negative coefficient of the interaction term. Collectively, these findings suggest that radiation exposure and subsequent metabolic profile changes, potentially in relationship to obesity-related inflammation, lead to such long-term alterations in lymphocyte subset composition. Because this study is based on cross-sectional and exploratory analyses, the implications regarding radiation exposure, metabolic profiles and circulating lymphocytes warrant future longitudinal and molecular mechanistic studies.

INTRODUCTION

Cells in the immune system are sensitive to ionizing radiation. For this reason, at the time of the atomic bombings over 70 years ago, the hematopoietic lineage cells in atomic bomb (A-bomb) survivors were assumed to be damaged by radiation in a dose-dependent manner. Although their damaged immune systems largely recovered within a few months (*1, 2*), radiation dose-associated impairments in their systems, reflected by the composition and function of circulating lymphocytes, are still observed even today (*3, 4*). Such lymphocyte changes associated with A-bomb radiation exposure include: 1. A decrease in the total CD4 T-cell population; 2. Decreases in naïve CD4 and naïve CD8 T-cell populations; 3. Reduced T-cell proliferation and interleukin (IL)-2 production; 4. An increase in the B-cell population; however, 5. No change in natural killer (NK) cell number or function.

Because the composition of peripheral blood lymphocytes clearly changes with aging and differs between men and women (*5–7*), age and gender were included in previous analyses examining lymphocyte subsets of A-bomb survivors (*3, 4*). However, it was recently recognized that several lifestyle factors and diseases, in particular, obesity-related metabolic profiles of individuals, were closely associated with the composition of circulating lymphocyte subpopulations (*8, 9*). In general, both CD4 T- and B-cell counts and percentages are higher in obese individuals than in lean individuals, and these increases are associated with risk factors of metabolic diseases, e.g., higher triglyceride and lower high-density lipoprotein (HDL) cholesterol levels. It

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has also been reported that peripheral T cells in obese individuals are somewhat activated, displaying increased expression levels of surface CD25 (a subunit of the IL2 receptor) and polarization towards a pro-inflammatory type-1 (T_H1) phenotype (*10–12*). It should be noted here that metabolic abnormalities, including serum levels of high triglyceride, low HDL cholesterol and fatty liver development, have been reported in association with radiation dose among A-bomb survivors (*13–15*). Moreover, emerging evidence from mouse studies suggests direct interactions between diet-induced obesity and biological responses to radiation, e.g., DNA double-strand breaks, microRNA expression and cell survival (*16, 17*). Therefore, lymphocyte subsets in A-bomb survivors may be influenced by their metabolic profiles especially after radiation exposure.

We previously examined the percentages of various lymphocytes (T-, B- and NK-cell subsets) in approximately 3,800 A-bomb survivors who underwent a health examination between 2000–2002, in relationship to hepatitis C virus (HCV) infection (*18*). The Adult Health Study (AHS) at the Radiation Effects Research Foundation (RERF) provided the individual metabolic profiles of the A-bomb survivors in the current study. These profiles consisted of body mass index (BMI), total serum cholesterol, HDL cholesterol, hemoglobin A1c (HbA1c) and C-reactive protein (CRP) levels as well as history of type-2 diabetes and fatty liver disease at the time of examination of lymphocyte subsets (*19, 20*). To investigate the hypothesis that the long-term effects of radiation on lymphocyte subsets may be modified by the metabolic profiles of individuals, the current study examined the relationships between the proportions of lymphocyte subsets in the circulation, radiation dose and indicators of obesity-related conditions or diseases in 3,113 A-bomb survivors.

MATERIALS AND METHODS

Study Population

Peripheral blood lymphocyte subsets were examined in almost all members of the AHS population (*19, 20*) who underwent health examinations at RERF outpatient clinics in Hiroshima and Nagasaki between 2000–2002 and whose radiation dose estimates were available (*21*). In addition, subjects entirely overlapped with those from a previous study (*18*), where lymphocyte subsets were separately analyzed in each of the following groups: an HCV-persistent infection group, an HCV-spontaneous clearance group and an HCV-uninfected group. In the current study, the HCV-spontaneous clearance group and the HCV-uninfected group were combined and included in the analyses, while persons in the HCV-persistent infection group were excluded because persistent HCV infection was found to profoundly impact the lymphocyte composition of peripheral blood. This study was approved by the RERF Institutional Review Board and the Human Investigation Committee, and all subjects provided written informed consent for inclusion prior to the examinations.

Lymphocyte Subset Data

For assessments of the changes in lymphocyte composition that are potentially associated with the metabolic profiles of A-bomb survivors, the current study used lymphocyte subset data that were mostly reported in a study published elsewhere (*18*), where methods for the lymphocyte subset analyses are fully described. In brief, flow cytometry-based analyses of circulating lymphocytes were performed using FACScan™ (BD Biosciences, San Jose, CA). The percentages of lymphocyte subsets (total T cells defined as $CD3^+$; CD4 T cells defined as $CD3^+CD4^+$; CD8 T cells defined as $CD3^+CD8^+$; naïve CD4 T cells defined as $CD3^+CD4^+CD45RA^+$; T_H1 cells defined as $CD3^+CD4^+CXCR3^+$; T_H2 cells defined as $CD3^+CD4^+CRTH2^+$; B cells defined as $CD3^-CD20^+$; and NK cells defined as $CD3^-CD16^+$) in the peripheral blood lymphocyte fraction were enumerated, as described in a previous study (*18*), where the focus was placed on effector and memory T-cell subsets; naïve CD4 T cells were not subjected to statistical analysis. In addition, the percentages within CD4 T cells were used for reporting T_H1 and T_H2 cells.

Clinical Data and Information on Lifestyle/Environmental Factors

Clinical blood examination data (total serum cholesterol, HDL cholesterol, HbA1c and CRP levels), BMI and disease information were obtained at the AHS examinations in 2000–2002 along with medical chart reviews, and diseases were classified according to International Classification of Diseases (ICD) codes, e.g., type-2 diabetes (E11–E14, based on blood HbA1c and glucose levels as well as treatment information such as hypoglycemic drugs or insulin) and fatty liver disease (K70.0 and K76.0, based on abdominal ultrasonography findings). Information on alcohol consumption and the number of cigarettes smoked was obtained from questionnaires at the time of the AHS examinations in 1993–1995 and 2000–2002, respectively. An indicator for smoking history over the past 10 years (current, former or never smokers) was also used in the analysis. In addition to these lifestyle factors, history of autoimmunity ($N = 293$), allergy ($N = 128$) and cancer ($N = 621$) prior to blood collection were used as adjustment factors in the analysis because they are known to influence lymphocyte subset composition (*6, 22, 23*). Radiation dose was estimated by the Dosimetry System 2002 (DS02) (*24*) based on the weighted bone marrow dose computed as the gamma dose plus 10 times the neutron dose (RERF Update 24: 27-29, 2013; RERF Update 25: 31-33, 2014: http://www.rerf.or.jp/library/update/index_e.html).

Statistical Data Analysis

The associations between lymphocyte subset percentages and age, gender, city, radiation dose and other variables were evaluated using standard multiple linear regressions (*25*) with the lymphocyte subset percentages log-transformed (base 10). The covariates used as potential confounders were: converted age (age −70) at the time of examination, gender (0 for male and 1 for female), city (0 for Hiroshima and 1 for Nagasaki), alcohol consumption (grams of ethanol/day), smoking amount (20 cigarettes/day) or smoking history (current or former smokers with reference to never smokers), autoimmune disease (1 if diagnosed, otherwise 0), allergic disease (1 if diagnosed, otherwise 0) and cancer (1 if diagnosed, otherwise 0). The covariates mainly used as potential radiation effect modifiers were: BMI (kg/m^2), total cholesterol (mg/dl), HDL cholesterol (mg/dl), CRP (mg/dl), HbA1c (%), type-2 diabetes (1 if diagnosed, otherwise 0) and fatty liver disease (1 if diagnosed, otherwise 0). The continuous variables (BMI, total cholesterol, HDL cholesterol, CRP and HbA1c) were centered to the median values and the centered values were used in regression analyses instead of the original values. Additionally, the centered values of total cholesterol and HDL cholesterol were divided by 100 to avoid very small coefficients.

Linear regression analyses of the log-transformed lymphocyte subset percentages were then performed using several models: model 1 (explanatory variables: age, gender, radiation dose, city, alcohol consumption, smoking and BMI); model 2 (age, gender, radiation dose, city, alcohol consumption, smoking, BMI, autoimmunity, allergy and cancer); and a basic model (only age, gender, radiation dose and city). Associations between lymphocyte subsets and

TABLE 1
Associations of Metabolic Indicators with T-, B- and NK-Cell Percentages

Regression analyses by cell type[a]	Coefficient	SE[b]	*P* value
T cells			
HDL cholesterol	–0.020	0.0076	0.01
CD4 T cells			
None			
Naive CD4 T cells			
BMI	–0.003	0.0015	0.03
Total cholesterol	0.030	0.0147	0.040
HDL cholesterol	0.134	0.0303	<0.001
Diabetes	–0.039	0.0138	0.005
T_H1 cells			
BMI	0.006	0.0009	<0.001
HDL cholesterol	–0.053	0.0183	0.004
Diabetes	0.033	0.0084	<0.001
Fatty liver	0.016	0.0064	0.02
T_H2 cells			
None			
CD8 T cells			
HDL cholesterol	–0.092	0.0196	<0.001
B cells			
BMI	0.006	0.0011	<0.001
HDL cholesterol	–0.079	0.0238	0.001
CRP[c]	–0.014	0.0063	0.03
HbA1c[d]	0.013	0.0045	0.004
Diabetes	0.025	0.0109	0.02
Fatty liver	0.023	0.0084	0.005
NK cells			
HDL cholesterol	0.104	0.0252	<0.001
HbA1c	–0.012	0.0047	0.012

[a] Age, gender, city, radiation dose, alcohol consumption, smoking, autoimmunity, allergy and cancer were adjusted in each regression analysis using a single metabolic indicator. Metabolic indicators are shown when $P < 0.05$.
[b] Standard error.
[c] CRP = C-reactive protein.
[d] HbA1c = hemoglobin A1c.

metabolic indicators were examined in model 2 with the change that BMI was replaced by one of the other metabolic indicators: total cholesterol, HDL cholesterol, CRP, HbA1c, diabetes or fatty liver. For model 3, metabolic indicators with $P < 0.05$ in these analyses were selected and used with age, gender, radiation dose, city, alcohol consumption, smoking, autoimmunity, allergy and cancer (i.e., the variables in model 2 plus selected metabolic indicators). In addition, the highest correlation coefficient for any two metabolic indicators was 0.70 (absolute value) between diabetes and HbA1c. The second and third highest correlation coefficients were 0.25 and 0.22 between total cholesterol and HDL cholesterol and between BMI and HDL cholesterol, respectively, which showed weak correlations. All other correlation coefficients were lower than 0.2. Because the relationship between diabetes and HbA1c is well established in medicine, these two strongly-correlated variables were used separately in model 3.

To investigate the multiplicative interaction effects between radiation exposure and metabolic profiles on lymphocyte subsets, we examined the association of interaction terms between radiation dose and each of the selected metabolic indicators (see Table 1) with lymphocyte cell percentages.

Hypothesis testing was based on Wald statistics and two-sided *P* values (with significance level of 0.05) are presented. We also considered the correction for multiple testing using the method described by Benjamini and Hochberg (*26*). All analyses were conducted using Stata software (Stata/SE 9.2 for Windows; StataCorp LP, College Station, TX).

RESULTS

Metabolic Profiles and Lymphocyte Subsets in A-Bomb Survivors

Table 2 shows the basic characteristics of the 3,113 study subjects. We first evaluated the relationships between metabolic profiles and lymphocyte subsets among the A-bomb survivors, using each of the variables related to metabolic conditions and diseases (BMI, total cholesterol, HDL cholesterol, CRP, HbA1c, type-2 diabetes and fatty liver) in a statistical model adjusted for age, gender, radiation dose, city, alcohol consumption, smoking, autoimmunity, allergy and cancer. Multiple metabolic indicators were significantly associated with naïve CD4 T-, T_H1-, B- and NK-cell populations (Table 1). Overall, indicators for obesity and related diseases were associated with larger percentages of the total T-, T_H1-, CD8 T- and B-cell populations, whereas such obesity indicators were associated with smaller percentages of the naïve CD4 T- or NK-cell populations. These results are consistent with our previous findings and those of other studies (*9, 27*). Particularly in this study, HDL cholesterol levels were closely associated with most cell subsets examined, displaying inverse associations with T, T_H1, CD8 T or B cells and positive associations with naïve CD4 T or NK cells. Although correction of multiple testing may not be appropriate in this study due to the interdependence among compared variables, when multiple testing was considered (seven regression analyses on eight cell types; overall $P = 0.05$), the following associations remained significant: HDL cholesterol with T cells, HDL cholesterol and diabetes with naïve CD4 T cells, BMI, HDL cholesterol and diabetes with T_H1 cells, HDL cholesterol with CD8 T cells, BMI, HDL cholesterol, HbA1c and fatty liver with B cells and HDL cholesterol with NK cells. In addition to the regression analyses including only one metabolic indicator (see Table 1), we also conducted analyses that included all six of the metabolic indicators (Supplementary Table S1; http://dx.doi.org/10.1667/RR14336.1.S1); the significance of most of the metabolic indicators became weaker, presumably due to the dependence among the variables. Indicators with significance ($P < 0.05$) in Supplementary Table S1 correspond mostly to those that remain significant after considering multiple testing in Table 1, being more directly related to lymphocyte subset composition than those that became nonsignificant after such multiple testing.

Radiation Exposure and Aging Effects on Lymphocyte Subsets

Effects of radiation dose and age on lymphocyte subsets were evaluated, using several statistical models (basic model and models 1–3), where covariates were employed

TABLE 2
Basic Characteristics of Study Subjects (N = 3,113)

Characteristic	Median (5th–95th percentile)	No. (percentage of study population)
Median age at time of examination	72.1 years (58.2–87.6)	
Median age at time of bombing	16.5 years (2.6–32.1)	
Gender		
No. of males		963 (30.9%)
No. of females		2,150 (69.1%)
Median radiation dose	0.077 Gy (0–1.777)	
No. of subjects by city		
Hiroshima		1,941 (62.4%)
Nagasaki		1,172 (37.6%)
Median alcohol consumption	0 g/day (0–70.5)	
No. of subjects by alcohol consumption		
0 g/day		1,861 (59.8%)
0–20 g/day		544 (17.5%)
20–40 g/day		298 (9.6%)
40–60 g/day		130 (4.2%)
>60 g/day		277 (8.9%)
Smoking		
Median number of cigarettes/day	0 (0–20.0)	
Smoking status		
No. of current smokers		409 (13.1%)
No. of former smokers		318 (10.2%)
No. of those who never smoked		2385 (76.6%)
BMI		
Median BMI	22.8 kg/m^2 (17.6–28.8)	
No. of subjects by BMI		
≤19.5 kg/m^2		454 (15.5%)
19.5–21.2 kg/m^2		474 (16.2%)
21.2–22.9 kg/m^2		602 (20.5%)
22.9–25.0 kg/m^2		659 (22.5%)
>25.0 kg/m^2		745 (25.4%)
Median total cholesterol	208.0 mg/dl (153.7–266.0)	
Median HDL cholesterol	60.5 mg/dl (38.2–93.0)	
Median HbA1c[a]	5.7% (4.9–7.6)	
Median CRP[b]	0.060 mg/dl (0.000–0.744)	
No. of subjects with diabetes		469 (15.1%)
No. of subjects with fatty liver		1,016 (32.6%)

[a] HbA1c = hemoglobin A1c.
[b] CRP = C-reactive protein.

as potential confounders of the radiation effects. In model 3, based on the results of the relationships between metabolic profiles and lymphocyte subsets, metabolic indicators whose relationships to lymphocyte subsets were at the level of $P < 0.05$ (shown in Table 1) were selected for statistical analyses. Table 3 summarizes the associations of lymphocyte subset percentages with age, gender and radiation dose, after adjustment for the selected metabolic indicators. Age- and radiation dose-dependent decreases were suggested in total T-, CD4 T- and naïve CD4 T-cell percentages, whereas T_H1- and T_H2-cell percentages tended to increase with both age and radiation dose. In a manner similar to that of total CD4 T cells, decreases in the B-cell population were age dependent, but no significant effects of A-bomb radiation were observed. Results regarding T- and B-cell subsets were nearly identical to those obtained in the simpler models, i.e., models 1, 2 and basic model (data not shown), but an effect of radiation dose on the NK-cell percentage was suggested (coefficient 0.013, $P = 0.043$) only in model 3 when the percentage was adjusted for metabolic indicators (in this case, HDL cholesterol and HbA1c). Fairly similar results of the main effects of radiation were obtained when adjustment was made for HDL cholesterol and diabetes, instead of HbA1c (coefficient 0.013, $P = 0.046$) and adjustment was made for only HDL cholesterol (coefficient 0.013, $P = 0.038$).

Interaction Effects between Radiation Exposure and Metabolic Profiles

Metabolic profile indicators associated with lymphocyte subset percentages (Table 1) might be potential modifiers of the radiation effects on lymphocyte subsets, because biological interactions between radiation responses and metabolic conditions were demonstrated in mouse studies (*16, 17*). To test this hypothesis, we investigated the association of the interaction term between radiation dose and each of the metabolic indicators whose associations with lymphocyte subsets were significant (see Table 1). No

TABLE 3
Associations of Age, Gender and Radiation Dose with T-, B- and NK-Cell Percentages

Regression analyses by cell type	Model 3[a] Coefficient	SE[b]	*P* value
T cells (additionally adjusted for HDL cholesterol)			
Age	–0.002	0.0002	<0.001
Gender	0.026	0.0034	<0.001
Radiation dose	–0.005	0.0020	0.005
CD4 T cells (no metabolic indicators were adjusted)			
Age	–0.003	0.0002	<0.001
Gender	0.034	0.0045	<0.001
Radiation dose	–0.013	0.0027	<0.001
Naive CD4 T cells (additionally adjusted for BMI, total cholesterol and HDL cholesterol)			
Age	–0.009	0.0007	<0.001
Gender	0.038	0.0136	0.005
Radiation dose	–0.033	0.0079	<0.001
T_H1 cells (additionally adjusted for BMI, HDL cholesterol and fatty liver)			
Age	0.001	0.0004	0.001
Gender	0.043	0.0083	<0.001
Radiation dose	0.011	0.0048	0.02
T_H2 cells (no metabolic indicators were adjusted)			
Age	0.002	0.0006	<0.001
Gender	–0.054	0.0129	<0.001
Radiation dose	0.013	0.0076	0.09
CD8 T cells (additionally adjusted for HDL cholesterol)			
Age	0.000	0.0004	0.700
Gender	0.020	0.0087	0.02
Radiation dose	0.009	0.0050	0.07
B cells (additionally adjusted for BMI, HDL cholesterol, CRP, HbA1c and fatty liver)			
Age	–0.006	0.0005	<0.001
Gender	0.112	0.0108	<0.001
Radiation dose	–0.007	0.0062	0.2
NK cells (additionally adjusted for HDL cholesterol and HbA1c)			
Age	0.010	0.0005	<0.001
Gender	–0.141	0.0114	<0.001
Radiation dose	0.013	0.0065	0.043

[a] Adjusted for city, alcohol consumption, smoking, autoimmunity, allergy, cancer and the metabolic indicators selected beforehand ($P < 0.05$) from six variables: BMI, total cholesterol, HDL cholesterol, CRP, HbA1c and fatty liver.
[b] Standard error.

significant interactions were observed in any T-cell subset examined (data not shown). However, we observed several interaction effects between radiation dose and BMI, CRP or fatty liver on the B-cell population and between radiation dose and HbA1c on the NK-cell population (Table 4). When correction of multiple testing was considered (18 tests; overall $P = 0.05$), only the interaction term between radiation dose and CRP on B cells remained significant. In addition, analyses using the cell count data instead of the percentages or using a categorical variable of smoking history instead of the number of cigarettes showed the same results regarding relationships between metabolic profiles and lymphocyte subsets, radiation effects on lymphocytes and interaction effects between radiation exposure and metabolic profiles (data not shown).

TABLE 4
Associations of Interaction Terms between Radiation Dose and Metabolic Indicators with B and NK Cells

Regression analyses by cell type[a]	Coefficient	SE[b]	*P* value
B cells			
Age	–0.006	0.0005	<0.001
Gender	0.104	0.0103	<0.001
Radiation dose	–0.003	0.0061	0.6
BMI	0.005	0.0014	0.001
Interaction: radiation dose × BMI	0.004	0.0018	0.038
B cells			
Age	–0.007	0.0005	<0.001
Gender	0.106	0.0103	<0.001
Radiation dose	–0.002	0.0065	0.8
CRP[c]	–0.001	0.0074	0.9
Interaction: radiation dose × CRP	–0.035	0.0104	0.001
B cells			
Age	–0.007	0.0005	<0.001
Gender	0.108	0.0103	<0.001
Radiation dose	–0.022	0.0077	0.004
Fatty liver	0.010	0.0099	0.3
Interaction: radiation dose × fatty liver	0.031	0.0124	0.011
NK cells			
Age	0.010	0.0005	<0.001
Gender	–0.132	0.0112	<0.001
Radiation dose	0.007	0.0068	0.3
HbA1c[d]	–0.019	0.0055	0.001
Interaction: radiation dose × HbA1c	0.015	0.0062	0.020

[a] Adjusted for city, alcohol consumption, smoking, autoimmunity, allergy and cancer. Regression analyses are shown for interaction terms with $P < 0.05$.
[b] Standard error.
[c] CRP = C-reactive protein.
[d] HbA1c = hemoglobin A1c.

DISCUSSION

In this human population study, we analyzed the effects of age, gender and radiation exposure on the circulating lymphocyte composition in conventional statistical models including lifestyle factors and several diseases. The results obtained were in line with those of previous studies of A-bomb survivors (*3, 18, 28, 29*), which have shown the remarkable radiation-associated feature of a decrease in the naïve CD4 T-cell number, prompting the hypothesis of radiation-accelerated T-cell immunosenescence (*4*). In this study, the decrease in the naïve CD4 T-cell number was also associated with indicators for obesity and related diseases such as higher BMI and lower HDL cholesterol levels. We have previously reported that T-cell production ability, assessed using T-cell receptor excision circles, is reduced along with increases in obesity indicators and serum CRP levels in A-bomb survivors (*27*). Mouse studies have suggested that obesity can cause T-cell production to become compromised through increases in adipocytes and their secreted inflammatory cytokines within the thymus and bone marrow (*30, 31*). Thus, both radiation exposure

and the obesity-related metabolic profile, potentially related to enhanced inflammation, are considered as accelerators of human T-cell immunological aging.

On the other hand, this study observed higher percentages of total T, T_H1, CD8 T and B cells along with obesity-related metabolic indicators, especially with low HDL cholesterol; these results are generally in accord with those from other human studies (*10–12, 32–37*). In animal models, a direct link between low HDL cholesterol levels and the vast expansion of various types of immune cells (T and B cells, macrophages and dendritic cells) has been established (*38*): A postulated mechanism is that HDL reduces cholesterol content in membrane lipid rafts, which can suppress intracellular signaling in such immune cells (*38*). Prolonged activation of T and B cells could also be an important mechanism by which aging perturbs immune cell homeostasis (*39*).

Low HDL cholesterol and fatty liver development have been reported to be associated with radiation dose among A-bomb survivors (*14*). Although causal relationships cannot be determined from the cross-sectional analyses of the current study, it can be hypothesized that the maintenance of peripheral lymphocyte populations may have been affected by an unidentified pathway that could involve radiation-associated alterations in metabolic profiles, in particular altered lipid metabolism. Specifically, HDL cholesterol was positively associated with the NK-cell percentage (Table 1), implying that the NK-cell percentage would be reduced by a radiation-associated lipid metabolism change, i.e., lowered HDL cholesterol. On the other hand, the regression analysis adjusted for HDL cholesterol showed that the NK-cell percentage was positively associated with radiation dose. These data may suggest that there are at least two pathways of radiation effects with opposite direction of action on the NK-cell percentage; one pathway is via altered metabolic profiles, and the other pathway is presumably related to a direct impact on the hematopoietic system. That may be the reason that the main effects of radiation on the NK-cell percentage appeared after adjusting for the metabolic indicators, resulting in an increase in NK cells with both age and radiation dose (Table 3). Thus, these results are consistent with our hypothesis that the long-term effects of radiation on lymphocyte subsets may be modified by metabolic profiles of individuals. The evolving hypothesis about mechanisms of radiation immune perturbation is therefore that altered immune cell composition after radiation exposure may be partly mediated by altered lipid metabolism. Subsequent studies, potentially contributing to a mechanistic understanding of the radiation effect modification, are needed to further test these hypotheses.

An interaction effect between radiation dose and CRP on the B-cell percentage that had a negative coefficient was observed (Table 4), suggesting that a combination of exposure to higher radiation doses and enhanced inflammation may lead to a decrease in the B-cell percentage. Additionally, the main effect of radiation on the B-cell percentage was also found in statistical models that included interaction terms between radiation dose and fatty liver development. It appears that the B-cell proportion decreases with radiation dose in subjects without fatty liver, whereas it increases with radiation dose in subjects diagnosed with fatty liver. However, this interaction effect with fatty liver was not statistically significant after consideration of the multiple testing correction.

In addition, in analyses shown in Tables 1, 3, 4, cell subset percentages within lymphocytes are used, constraining correlations among total T-, B- and NK-cell percentages as well as between naïve CD4 T-, T_H1- and T_H2-cell percentages. It is likely that, for example, increased T_H1- and T_H2-cell percentages with radiation dose are related to decreased naïve CD4 T-cell percentages. However, the results of Tables 1, 3, 4, are similar to those from analyses using cell counts/μl of peripheral blood; the correlations of the cell subset percentages do not affect our main findings such as reduced naive CD4 T cells and increased NK cells in association with radiation dose or interaction effects between radiation dose and metabolic profiles on B cells.

In a previously reported study, the B-cell proportion was observed to increase in A-bomb survivors who were heavily exposed to radiation (*40*), although the number of subjects ($N = 411$) was much smaller than in the current study and the metabolic profiles of the subjects were not considered. A limitation of the current study may thus involve the heterogeneity of the peripheral B-cell population, which was not considered. Radiation exposure and metabolic status may differentially influence distinct B-cell subpopulations (*5, 40, 41*), i.e., immature or mature B cells and functionally different subsets of B cells, such as follicular (conventional), marginal zone and B-1 B cells. Therefore, a potential way to clarify the biological significance of the interactions observed in this study would be to assess a more diverse subset of B cells, as specific subsets may prove to be more closely associated with radiation exposure or metabolic profiles.

Because the current study is based on cross-sectional analyses with an exploratory nature, a future assessment using a longitudinal study design combined with detailed cellular and molecular analyses is necessary to verify the relationships between radiation exposure, metabolic profiles and circulating lymphocyte subsets. Although increases in T_H1- and B-cell populations may be implicated in diabetes and fatty liver development (Table 1), how and to what extent these lymphocytes contribute to disease development should also be investigated in future longitudinal and mechanistic studies. Such studies are actually feasible, using cryopreserved lymphocytes at RERF, which have been repeatedly collected from approximately 9,000 AHS participants over a period of 20 years.

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