TABLE 1
Characterization of Study Population

	Age (years)[b]								
	<60		60–69		70–79		80+		
Dose (Gy)	Male	Female	Male	Female	Male	Female	Male	Female	Total
<0.005[a]	11	11	23	16	20	41	12	19	153
0.005–0.5	5	5	10	21	23	28	18	20	130
0.5–1.0	9	6	15	25	23	29	7	12	126
1.0–4.0	13	9	15	21	23	26	5	12	124
Total	38	31	63	83	89	124	42	63	533

[a] Individuals in this dose category were exposed at distances in excess of 3 km from the hypocenter and hence received doses that are functionally equivalent to zero.
[b] Age at the time of the examinations, which were conducted between September 2000 and February 2003.

of A-bomb survivors. Flow cytometry using CD62L marker not only allowed precise detection of naïve T cells but also enabled us to determine the percentages of memory T-cell subpopulations in PBL of A-bomb survivors, demonstrating dose-dependent increases in the percentages of $CD45RO^+/CD62L^+$ and $CD45RO^+/CD62L^-$ memory CD8 T cells.

MATERIALS AND METHODS

Study Population

Blood samples were obtained from individual members of an A-bomb survivor cohort in which 1,280 survivors, distributed almost equally by age, gender and radiation dose, had been selected from Hiroshima participants in the Adult Health Study (AHS) at the Radiation Effects Research Foundation (RERF) in 1992 (*3*). The selected study population consists of Hiroshima survivors who were exposed to significant radiation doses of 0.005 Gy or more because of their location within 2 km of the hypocenter plus a second group whose exposures were at distances in excess of 3 km from the hypocenter and as a result would have received less than 0.005 Gy (i.e. would have been exposed to doses that are indistinguishable from background). The latter group of distally exposed survivors includes the most appropriate controls for all of our studies of the effects of A-bomb radiation exposures, including this one. The estimated radiation doses are based on DS86 with the adjustments that are standard practice at RERF (*15, 16*). Of these individuals, 653 reported for examination between September 2000 and February 2003 (see *Flow Cytometry and Schedule of Measurements* below). We decided to exclude results from 120 of these 653 people who had been diagnosed with cancer from our study. The age, gender and radiation dose of the remaining 533 survivors whose lymphocyte samples were subjected to detailed analysis in our study are listed in Table 1. Blood samples were obtained with the informed consent of the survivors, and PBL were separated by the Ficoll-Hypaque gradient technique (*7*).

In Japan, projects of this type must obtain approval from the appropriate Institutional Ethics Committee prior to the commencement of work. In the case of RERF, the relevant committee at the time this project was carried out was known as the Human Investigation Committee, and its approval was sought and granted before the work was started.

Monoclonal Antibodies (mAbs)

FITC-labeled anti-CD4, biotin-labeled anti-CD4, PerCP-labeled anti-CD4, biotin-labeled anti-CD8, PerCP-labeled anti-CD8, phycoerythrin (PE)-labeled anti-CD8, PE-labeled anti-CD3, PerCP-labeled anti-CD3, FITC-labeled anti-CD57, FITC-labeled anti-TCRαβ, and PE-labeled anti-CD62L mAbs were purchased from BD PharMingen (San Diego, CA). PE-labeled anti-CD45RA mAb and FITC-labeled anti-CD45RO mAb were obtained from Coulter Immunotech (Marseille, France) and Caltag Laboratories (Burlingame, CA), respectively.

Flow Cytometry and Schedule of Measurements

Analytical flow cytometry was conducted in a FACScan machine (BD Biosciences, San Jose, CA). Between September 2000 and February 2003, expression of CD45RO and CD62L was analyzed using a combination of FITC-labeled anti-CD45RO, PE-labeled anti-CD62L, and PerCP-labeled anti-CD4 or PerCP-labeled anti-CD8 mAbs. $CD45RO^-/CD62L^+$, $CD45RO^+/CD62L^+$, $CD45RO^+/CD62L^-$ and $CD45RO^-/CD62L^-$ cell fractions in CD4 and CD8 T-cell populations were determined as shown in Fig. 1. Note that we used only bright CD8 expression to identify CD8 T cells to exclude NK cells, which are dully CD8 positive. The percentages of CD45RA-positive naïve and CD45RA-negative memory CD4 T cells in the PBL fractions were measured between October 1992 and March 1995 (*3*). Analyses of the expression of CD45RA in the CD8 T-cell subset involved staining mononuclear cell fractions with FITC-labeled anti-TCRαβ mAb and PE-labeled anti-CD45RA mAb, and with biotin-labeled anti-CD8 mAb sandwiched by streptavidin-RED670 (Gibco BRL, Rockville, MD); the CD45RA expression was determined for the CD8 and TCRαβ double-positive fraction of the survivors' PBL between October 1992 and March 1995. From April 1997 to April 1999, the expression of CD57 in the CD8 T-cell subsets was analyzed using a combination of PE-labeled anti-CD3, PerCP-labeled anti-CD8, and FITC-labeled anti-CD57mAbs; the CD57 expression was determined for the CD3 and CD8 double-positive fraction. The expression of CD28 in the CD8 T-cell subset was analyzed using a combination of PerCP-labeled anti-CD3, PE-labeled anti-CD8, and FITC-labeled anti-CD28mAbs, and the CD28 expression was determined for the CD3 and CD8 double-positive fraction from May 1999 to April 2001. In every measurement, approximately 20,000 cells were analyzed.

Data Analysis

We used a set of data obtained from individuals whose PBL samples were subjected to examinations of CD45RO and CD62L expression to describe the T-cell subpopulations of all 533 A-bomb survivors. Associations of the percentage of each T-cell subpopulation (*percentage*) with age at the time of examination (*age*), gender and radiation dose (*dose*) were analyzed based on the following multiple regression model (*17*), assuming that the percentage of each T-cell subpopulation was related to each explanatory variable in an exponential manner:

$$\log(percentage) = \alpha + \beta_1 age + \beta_2 gender + \beta_3 dose,$$

where

$$gender = \begin{cases} 0 & \text{for male and} \\ 1 & \text{for female} \end{cases}$$

TABLE 2
Regression Coefficients for Variables Related to the Percentages of CD4 and CD8 T-Cell Subpopulations Expressing Different CD45 Isoforms in PBL among A-Bomb Survivors[a]

Duration of measurements	T-cell subpopulation	Intercept α	Age (10 years)[b] β_1	Gender[c] β_2	Dose (Gy)[d] β_3
September 2000 to February 2003	CD4				
	CD45RO$^-$	4.61	−0.260 $P = 0.0001$**	0.034 $P = 0.56$	−0.083 $P = 0.036$*
	CD45RO$^+$	3.43	−0.025 $P = 0.057$	0.084 $P = 0.0006$**	−0.002 $P = 0.92$
	CD8				
	CD45RO$^-$	2.45	−0.098 $P = 0.0008$**	0.102 $P = 0.064$	−0.063 $P = 0.098$
	CD45RO$^+$	1.98	0.027 $P = 0.27$	−0.077 $P = 0.091$	0.074 $P = 0.013$*
October 1992 to March 1995	CD4[e]				
	CD45RA$^+$	3.84	−0.124 $P = 0.0001$**	0.035 $P = 0.34$	−0.054 $P = 0.034$*
	CD45RA$^-$	2.94	0.007 $P = 0.63$	0.65 $P = 0.014$*	−0.016 $P = 0.35$
	CD8[f]				
	CD45RA$^+$	2.77	−0.065 $P = 0.0053$**	0.006 $P = 0.88$	0.004 $P = 0.88$
	CD45RA$^-$	1.04	0.022 $P = 0.49$	−0.182 $P = 0.0018$**	0.043 $P = 0.28$

[a] Regression coefficients of percentage T cells for age, gender and dose were obtained using the following formula: $Percentage\ T\ cells = \alpha + \beta_1 \times age + \beta_2 \times gender + \beta_3 \times dose$.
[b] Effects of age were estimated for 10-year intervals.
[c] Gender = 0 for male and = 1 for female.
[d] Effects of dose were estimated for 1 Gy.
[e] Results for a total of 723 A-bomb survivors have been reported in ref. (*3*), and those for 497 survivors whose data are available for both the current (2000–2003) and previous (1992–1995) studies are presented here.
[f] Results for 497 survivors whose data are available for both the current and previous studies are presented here.
* $P < 0.05$, ** $P < 0.01$.

RESULTS

Three-Color Flow Cytometry Involving CD62L Expression Revealed Effects of A-Bomb Radiation on Naïve CD8 T-Cell Population

Although a dose-dependent decrease in the percentage of naïve CD4 T cells that were enumerated with the CD45RA$^+$ phenotype has been observed repeatedly among A-bomb survivor populations (*3, 7*), radiation effects on naïve CD8 T-cell populations remained to be investigated. Table 2 summarizes the results of CD45RO expression analyses for a total of 533 A-bomb survivors who were examined between September 2000 and February 2003, along with the results of CD45RA expression analyses that were obtained from previous examinations (October 1992 to March 1995). We found high correlations between the current and previous examinations. The correlation coefficients (r) between the percentages of CD45RO$^-$ (current) and CD45RA$^+$ (previous) subsets in CD4 and CD8 T-cell populations were 0.85 ($P = 0.0001$) and 0.68 ($P = 0.0001$), respectively. Between the percentages of CD45RO$^+$ (current) and CD45RA$^-$ (previous) subsets in CD4 and CD8 T-cell populations, r was 0.75 ($P = 0.0001$) and 0.64 ($P = 0.0001$), respectively. This suggested that it would be reasonable to compare the effects of age, gender and radiation dose on these T-cell subsets in the previous and current examinations. As for CD4 T-cell populations, the effects of age, gender and radiation dose on CD45RO$^-$ and CD45RO$^+$ subsets appeared to be virtually in accord with those effects on CD45RA$^+$ and CD45RA$^-$ subsets. There appeared to be a significant effect of radiation on CD45RO$^+$ CD8 T cells in the current examination but not on the counterpart CD45RA$^-$ CD8 T cells in the previous study. At any rate, significant dose-dependent reductions in the percentages of CD45RO$^-$ and CD45RA$^+$ subsets containing naïve cells were apparent for the CD4 but not CD8 T-cell populations of the survivors, probably due to a substantial fraction of memory CD8 T cells included in the CD45RO$^-$ and CD45RA$^+$ subsets (*10, 18, 19*).

In the present study, we intended to discriminate naïve and memory cells in the CD45RO$^-$ subset by using CD62L as the third marker. Figure 1 shows representative flow cytometry patterns that were obtained from two typical male and female survivors who were relatively young and old. It is clear that the CD45RO$^-$ fraction of CD4 T-cell populations consists mostly of CD62L$^+$ cells, whereas there are

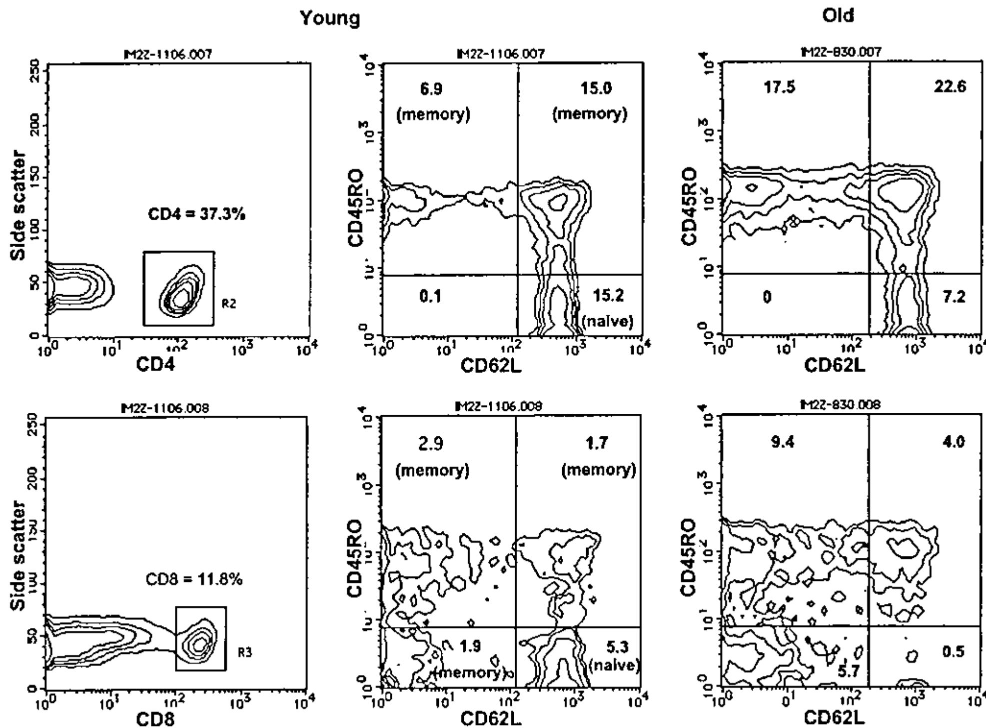

FIG. 1. Flow cytometry patterns of peripheral blood CD4 (upper panel) and CD8 (lower panel) T cells of typical A-bomb survivors who were relatively young (unexposed 58-year-old male, left and center columns) and old (unexposed 93-year-old female, right column), respectively. Peripheral blood mononuclear cells (about 2×10^5) were stained with FITC-labeled anti-CD62L mAb, PE-labeled anti-CD45RO mAb, and PerCP-labeled anti-CD4 mAb (upper panel) or PerCP-labeled anti-CD8 mAb (lower panel). The number in each quadrant indicates the percentage of cells in PBL.

substantial numbers of CD62L$^-$ cells in the CD45RO$^-$ fraction of CD8 T-cell populations. Although there were some individuals who had significant fractions of CD45RO$^-$/CD62L$^-$ cells in their CD4 T-cell populations, we did not obtain any evidence that A-bomb radiation had affected this minor T-cell subset in any obvious way (data not shown). The CD45RO$^+$ fractions of both CD4 and CD8 T-cell populations can also be divided into CD62L$^+$ and CD62L$^-$ subpopulations. Figure 2 depicts the age and dose trends for the percentage of CD45RO$^-$/CD62L$^+$ (naïve) cells in CD4 and CD8 T-cell populations. In the CD4 T-cell populations, the percentage of CD45RO$^-$/CD62L$^+$ (naïve) cells decreased significantly with increased age (25% per 10-year increment, $P = 0.0001$) or radiation dose (9% per gray, $P = 0.034$), almost concordant with that of CD45RA$^+$ or CD45RO$^-$ cells (also see Table 2). As had been expected, the decrease in the percentage of CD45RO$^-$/CD62L$^+$ (naïve) CD8 T cells was also statistically significant, i.e. a 35% decrease with a 10-year increment of age ($P = 0.0001$) and an 8% decrease per gray ($P = 0.031$). These results clearly indicate that the history of radiation exposure has generated a long-lasting reduction of naïve cell pools in both the CD4 and CD8 T-cell populations among A-bomb survivors.

Dose-Dependent Increases in the Percentages of Memory CD8 T-Cell Subsets

The percentage of CD45RO$^+$ CD8 memory T cells appeared to increase significantly with radiation dose (Table 2). This was not obvious in the measurement with CD45RA, which was used as a naïve/memory marker. To describe the memory T-cell subsets in this study, we determined two distinct CD62L$^+$ and CD62L$^-$ compartments in CD45RO$^+$ cells in both CD4 and CD8 T-cell populations, along with an additional CD62L$^-$ compartment in the CD45RO$^-$ CD8 T-cell population (Fig. 1). It has been reported that memory T cells in the CD62L$^+$ compartment have an increased potential to proliferate in response to recall antigens *in vitro* and a greater capacity to persist *in vivo* than memory T cells in the CD62L$^-$ compartment (*20, 21*). For CD4 but not CD8 T-cell populations, the percentages of CD45RO$^+$/CD62L$^+$ memory T cells in PBL were found to decrease significantly with increasing age of the A-bomb survivors (Fig. 3 and Table 3). We also found dose-dependent increases in the percentages of CD45RO$^+$/CD62L$^+$ CD8 T cells (12% increase per gray, $P = 0.0055$) and CD45RO$^+$/CD62L$^-$ CD8 T cells (8% increase per gray, $P = 0.034$), which were not found for the CD45RO$^+$/

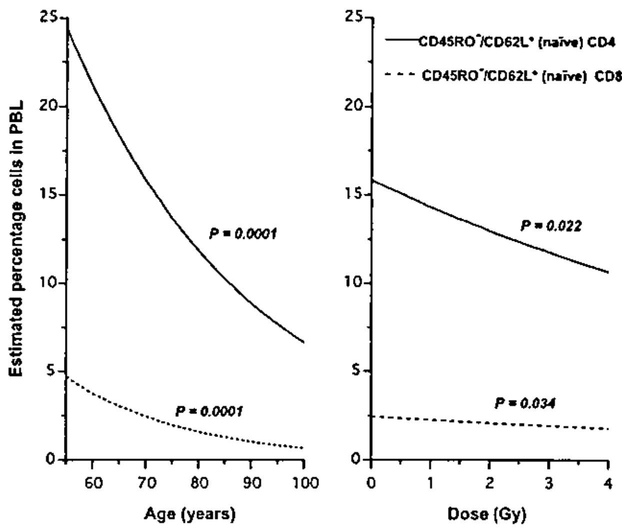

FIG. 2. Estimated radiation dose responses in the percentages of naïve (CD45RO$^-$/CD62L$^+$) CD4 and CD8 T cells in PBL among 533 A-bomb survivors. The values were adjusted to those of unexposed males or 70-year-old males and plotted as a function of age (left panel) or radiation dose (right panel), respectively, according to the formula described in the Materials and Methods.

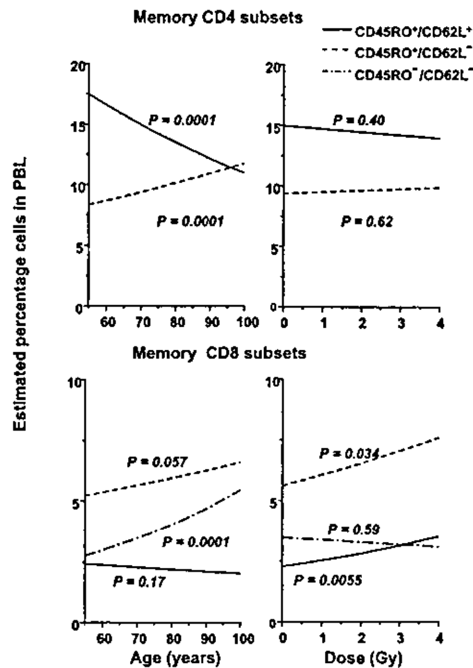

FIG. 3. Estimated radiation dose responses in the percentages of memory CD4 (upper panel) and CD8 (lower panel) T-cell subsets in PBL among 533 A-bomb survivors. The values were adjusted to those of unexposed males or 70-year-old males and plotted as a function of age (left panels) or radiation dose (right panels), respectively, according to the formula described in the Materials and Methods.

CD62L$^+$ or CD45RO$^+$/CD62L$^-$ CD4 T cells among the same survivors. There was no effect of dose on CD45RO$^-$/CD62L$^-$ cells in CD8 T-cell populations. These results indicate that the history of radiation exposure has dose-dependently caused increases in subsets (CD45RO$^+$/CD62L$^+$ and CD45RO$^+$/CD62L$^-$) of memory CD8 T cells among A-bomb survivors.

Increased Percentages of CD45RO$^+$/CD62L$^+$ or CD45RO$^+$/CD62L$^-$ CD8 T Cells by Radiation Exposure may not be Restricted toCD28$^-$ or CD57$^+$ T Cells

Other studies have reported that clonal expansion of a subset of CD8 T cells, such as CD28$^-$ or CD57$^+$, occurred frequently in older individuals (*22–25*). To test whether the increased percentage of CD45RO$^+$/CD62L$^+$ or CD45RO$^+$/CD62L$^-$ CD8 T cells that we observed among the survivors was associated with expansion of CD28-negative or CD57-positive cell populations, we analyzed the effects of age, gender and dose on the percentages of CD28$^-$ and CD57$^+$ CD8 T cells and examined the correlations among CD28$^-$, CD57$^+$, CD45RO$^+$/CD62L$^+$, and CD45RO$^+$/CD62L$^-$ CD8 T cells (Table 4). The percentages of CD28$^-$ and CD57$^+$ CD8 T cells were much less correlated with those of CD45RO$^+$/CD62L$^+$ CD8 T cells (r = 0.18 and 0.22, respectively) than those of CD45RO$^+$/CD62L$^-$ CD8 T cells (r = 0.62 and 0.60, respectively). This could indicate that expansion of CD45RO$^+$/CD62L$^-$ CD8 T cells is associated with increased proportions of CD28$^-$ or CD57$^+$ CD8 T cells. However, no significant effect of A-bomb radiation on CD28$^-$ or CD57$^+$ CD8 T cells was found, although there did appear to be a remarkable increase with age in the percentage of these subsets. Thus it is implied that the increased percentages of CD45RO$^+$/CD62L$^+$ and CD45RO$^+$/CD62L$^-$ CD8 T cells with increased radiation dose may not be largely due to increases in the percentages of CD28$^-$ and CD57$^+$ CD8 T cells.

DISCUSSION

Our present findings clearly indicate that radiation exposure has reduced the size of naïve cell pools not only in CD4 T-cell populations but also in the other major T-cell subset, CD8, among A-bomb survivors. One of the most plausible explanations for this is that the reduction in the size of naïve T-cell pools could have resulted from an insufficient supply of new T cells from the thymus since the majority of the naïve T cells develop in the thymus. Recently, it has been reported that the numbers of recent thymic emigrant cells can be determined in T-cell populations by detecting T lymphocytes that contain T-cell receptor-rearrangement excision circles (TRECs), which are circular DNA molecules generated in thymocytes during the T-cell receptor gene rearrangement process in the thymus (*26*). It has also been reported that significantly fewer CD4 T cells containing TRECs are currently demonstrable in bone marrow transplantation patients who were exposed to whole-body irradiation more than 20 years ago (*27*). Thus it is

TABLE 3
Regression Coefficients for Variables Related to the Percentages of CD4 and CD8 T-Cell Subpopulations Expressing Different Levels of CD62L in PBL among A-Bomb Survivors[a]

T-cell subpopulation	Intercept α	Effects: Age (10 years)[b] β_1	Gender[c] β_2	Dose (Gy)[d] β_3
CD4				
$CD45RO^-/CD62L^+$	4.79	−0.290 $P = 0.0001$**	0.022 $P = 0.72$	−0.099 $P = 0.022$*
$CD45RO^+/CD62L^+$	3.43	−0.104 $P = 0.0001$**	0.096 $P = 0.0037$**	−0.019 $P = 0.40$
$CD45RO^+/CD62L^-$	1.71	0.076 $P = 0.0001$**	0.070 $P = 0.046$*	0.012 $P = 0.62$
CD8				
$CD45RO^-/CD62L^+$	3.92	−0.432 $P = 0.0001$**	0.170 $P = 0.0020$**	−0.081 $P = 0.034$*
$CD45RO^+/CD62L^+$	1.11	−0.042 $P = 0.17$	0.025 $P = 0.66$	0.109 $P = 0.0055$**
$CD45RO^+/CD62L^-$	1.37	0.052 $P = 0.057$	−0.116 $P = 0.025$*	0.075 $P = 0.034$*
$CD45RO^-/CD62L^-$	0.188	0.151 $P = 0.0001$**	−0.007 $P = 0.93$	−0.027 $P = 0.59$

[a] Regression coefficients of percentage T cells for age, gender and dose were obtained using the following formula: *Percentage T cells* $= \alpha + \beta_1 \times age + \beta_2 \times gender + \beta_3 \times dose$.
[b] Effects of age were estimated for 10-year intervals.
[c] Gender = 0 for male and 1 for female.
[d] Effects of dose were estimated for 1 Gy.
* $P < 0.05$, ** $P < 0.01$.

expected that a similar reduction in the number of recent thymic emigrant cells would be observed among A-bomb survivor populations. Therefore, we recently started measuring the numbers of recent thymic emigrant cells that can be detected in both the CD4 and CD8 T-cell populations of A-bomb survivors with a view to determining whether a reduced production of new T cells in the thymus is an actual cause of the impaired maintenance of naïve T-cell pools among survivors. Another plausible mechanism is that the reduction of the naïve cell pool size might have resulted from the homeostatic and/or antigenic proliferation of naïve T-cell populations followed by their transfer to memory T-cell pools. We are also planning to analyze the turnover of naïve CD4 T-cell populations by tracking genetically marked murine naïve CD4 T cells after implantation into irradiated mice. Such an experimental model will provide us with valuable information for a precise understanding of what happens when radiation exposure seriously impairs the ability of irradiated hosts to maintain normal-sized naïve CD4 T-cell pools.

In most individuals, CD62L-negative cells are undetectable among $CD45RO^-$ CD4 T-cell populations while $CD45RO^-$ CD8 T-cell populations contain a significant fraction of CD62L-negative cells that are known to exhibit potential effector functions (*10–14*). We found that the percentages of $CD45RO^-/CD62L^-$ CD8 T cells increased with age (Fig. 3, Table 3). Although the meaning of this age dependence is unclear, it is not inconceivable that frequent antigen exposures due to decreased immunocompetence in aged individuals leads to an accumulation of effector T-cell populations.

Recent studies suggest that $CD45RA^-(CD45RO^+)$ memory T cells are heterogeneous in their functions and that they comprise distinct populations (*21, 22, 28–34*). These memory subsets can also be distinguished based on the expression of other surface markers, including CD62L (*21, 30, 32, 33*). In addition to impairment in the maintenance of naïve cell pools, our results also suggest that A-bomb radiation has induced a change in the memory T-cell pools of the survivors' CD8 T-cell populations that involves a dose-dependent increase in the percentage of $CD45RO^+/CD62L^+$ or $CD45RO^+/CD62L^-$ cells. Sallusto *et al.* (*28*) have recently proposed that memory ($CD45RA^-$ and $CD45RO^+$) T cells can be classified into central ($CCR7^+$ and mostly $CD62L^+$) and effector ($CCR7^-$ and mostly $CD62L^-$) memory T cells. These subsets were found to show several differences in their functions (*34*), migration capacities (*28*), proliferation abilities (*21, 22, 32*), telomere lengths (*21*), and T-cell receptor repertoires (*33*). These findings suggest that effector memory T cells are immature compared to central memory T cells (*35*), although it is unclear whether the latter memory subset stems from the former (*33, 36*).

Here we discuss possible reasons that the radiation effect is apparent for both the central and effector CD8 memory T-cell subsets. One possible interpretation is that the entry

TABLE 4
Regression Coefficients for Variables Related to the Percentages of CD28-Negative and CD57-Positive CD8 T-Cell Subpopulations and their Correlations to the Percentages of $CD45RO^+/CD62L^+$ and $CD45RO^+/CD62L^-$ CD8 T Cells in PBL among A-bomb Survivors[a]

T-cell subpopulation	Intercept α	Age (10 years)[b] β_1	Gender[c] β_2	Dose (Gy) β_3
$CD28^-$	1.23	0.177 $P = 0.0001$**	−0.072 $P = 0.25$	−0.016 $P = 0.70$
$CD57^+$	1.14	0.103 $P = 0.0048$**	−0.149 $P = 0.027$*	−0.033 $P = 0.48$

Correlation between	Correlation coefficients (r)
$CD28^-$ and $CD45RO^+/CD62L^+$	0.18 $P = 0.0001$**
$CD28^-$ and $CD45RO^+/CD62L^-$	0.62 $P = 0.0001$**
$CD57^+$ and $CD45RO^+/CD62L^+$	0.22 $P = 0.0001$**
$CD57^+$ and $CD45RO^+/CD62L^-$	0.60 $P = 0.0001$**
$CD28^-$ and $CD57^+$	0.84 $P = 0.0001$**

[a] Regression coefficients of percentage T cells for age, gender and dose were obtained using the following formula: $Percentage\ T\ cells = \alpha + \beta_1 \times age + \beta_2 \times gender + \beta_3 \times dose$.
[b] Effects of age were estimated for 10-year intervals.
[c] Gender = 0 for male and = 1 for female.
* $P < 0.05$, ** $P < 0.01$.

of naïve CD8 T cells into the memory T-cell pool is enhanced in A-bomb survivors. Repeated exposure of naïve CD8 T cells to various antigens could have led to accumulation of both central and effector memory T cells. Impaired immunity to persistent infections with viruses such as EBV (*37, 38*), HBV (*5, 39*) and HCV (*40, 41*) in A-bomb survivors might be involved in this accumulation process. Homeostatic proliferation that takes place in the absence of antigen under lymphopenic conditions such as radiation-induced lymphopenia may also have contributed to the enhanced entry of naïve T cells into memory T-cell pools. However, such a possibility may contradict a previous finding, in which a population of mutant stem cells of one A-bomb survivor did not contributed greatly to the maintenance of the survivor's memory T-cell pool after A-bomb exposure (*42*).

Another interpretation is that clonal expansions of a population of memory CD8 T cells have frequently occurred in A-bomb survivors. A recent cytogenetic study on A-bomb survivors has shown that the frequency of clonal chromosome aberrations increased with increasing radiation dose and suggested that about half of the clonal chromosome aberrations may have been derived from clonal expansions of memory T cells (*43*). It has also been found that skewed TCR Vβ repertoires that are possibly associated with clonal expansions can frequently be observed in $CD45RA^-$ memory CD4 T-cell populations of A-bomb survivors, especially in those of older survivors (*44*). Although we have not yet determined the TCR Vβ repertoires in CD8 T-cell populations of the survivors, similar expansions of memory CD8 T cells can be expected among radiation-exposed persons. Nevertheless, the dose-dependent increases in the percentages of $CD45RO^+/CD62L^+$ and $CD45RO^+/CD62L^-$ memory T cells were apparent for CD8 T-cell populations but not for CD4 T-cell populations, suggesting that clonal expansions frequently occurring in the exposed individuals might not accompany the accumulation of memory T cells.

In contrast to the dose-dependent increases in the percentages of $CD45RO^+/CD62L^+$ and $CD45RO^+/CD62L^-$ CD8 T cells, there was no significant effect of radiation on these subsets of memory CD4 T cells in the present study. Although it has been established that no antigenic stimulation is required for either the CD4 or CD8 T-cell populations to maintain immunological memory, the ways in which the memory T-cell pools maintain their sizes are likely to be somewhat different for CD4 and CD8 T-cell populations. Thus, for example, homeostatic proliferation of memory CD8 T cells is accomplished by IL7 and IL15, whereas unknown factors other than those cytokines are probably involved in that of memory CD4 T cells (*45, 46*). Memory CD8 T cells are likely to persist *in vivo* for much longer than memory CD4 T cells (*47*). Furthermore, age-associated reductions in the diversity of TCR repertoire were found to be much more pronounced in memory CD8 populations than memory CD4 populations (*48*). Such differences in the CD4 and CD8 T-cell populations may be associated with the different radiation effects we observed in the $CD45RO^+/CD62L^+$ and $CD45RO^+/CD62L^-$ subsets of these T-cell populations among A-bomb survivors. The mechanisms by which radiation exposure impairs the ability of humans to maintain T-cell memory remain to be clarified.

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The peritoneal mesothelium acts as a bioactive cellular membrane regulating serosal responses to injury, infection and neoplastic diseases. Inflammation of the serosal surfaces induces an 'activated' mesothelial cell phenotype. In the present study we simulated activation of cultured human peritoneal mesothelial cells (HPMC, isolated from human omentum majus) by treatment with the pro-inflammatory cytokine interleukin-1beta (IL-1beta).). Respiratory activity of suspended cells was analysed by high-resolution respirometry to assess changes in respiratory capacity and coupling of oxidative phosphorylation in activated HPMC. Citrate synthase (CS) and lactate dehydrogenase (LDH) activites were determined by spectrophotometry. IL-1beta activation for 48 h resulted in a significant decline of respiratory capacity ($P < 0.05$), without affecting integrity of the inner mitochondrial membrane or cell viability. Treatment of HPMC with IL-1beta resulted in a decrease of CS activity ($P < 0.05$) and an increase of LDH activity ($P < 0.05$). The present data indicate that activation of peritoneal mesothelial cells with IL-1beta is associated with a decrease of oxidative phosphorylation and mitochondrial content that appears to be compensated by an increase in glycolytic capacity.

Rapid detection of micrometastasis by one step nucleic acid amplification

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Introduction Lymph node metastasis is one of important prognostic factors of caner patients. We examined the efficacy of one step nucleic acid amplification (OSNA) for rapid detection of lymph node metastasis with GD-100 (SYSMEX).

Materials and methods Five colon cell lines (Lovo, DLD1, WiDr, Colo201, Colo320) and 11 lymph nodes of colorectal carcinoma were examined. Cytokeratin (CK) 19 mRNA of cell lines and lymph nodes were measured quantitatively by OSNA.

Results Cellular mRNA of Lovo, DLD1, WiDr and Colo201 differed from 85.7 to 113.2 copies at semiconfluent condition. CK19 mRNA tended to vary at the different cell density except for Colo320, in which no CK mRNA was detected. By using Lovo cells, 0.8 cells were calculated as the least detectable number at one reaction. In the lymph nodes analysis, OSNA was completed within 30 min and results by OSNA were equal to those by histological examination when whole lymph node was examined for both methods.

Conclusion OSNA is a newly developed useful method to detect micrometastasis rapidly.

Partially rescue of Cbfa1 deficiency by Nell-1 overexpression

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Introduction Overexpression of Nell-1 associates with craniosynostosis in both human patients and transgenic mice, while *in vitro* Nell-1 overexpression induces osteoblast differentiation and mineralization.[1] *Nell-1* promoter analysis suggests transcriptional regulation by Cbfa1, a critical mediator of bone formation.

Materials and methods To determine if Cbfa1 can induce *Nell-1* expression, mouse calvarial cells were transfected with pcDNA3.1-Cbfa1. *Nell-1* and *osteocalcin* expression were examined by RT-PCR. To determine if Nell-1 can functionally compensate for some aspects of Cbfa1 deficiency *ex vivo* and *in vivo*: (1) calvaria explants from newborn Cbfa1 ± haploinsufficient mice were treated with Nell-1 protein and bone mineralization monitored by calcein assay and skeleton staining and (2) mice$^{Cbfa1+/-}$ were intercrossed with mice$^{Nell-1\ overexp}$ and skeletal morphology analyzed by microCT and histopathology.

Results Cbfa-1 induced *Nell-1* mRNA as early as 6 h, and ostoecalcin by 12 h post-transfection. Mineralization in calvarial explants was significantly increased in Nell-1 protein treated specimens. Intercrossed progeny (e.g. mice*Nell-1 overexp, Cbfa1-/-*) manifested significantly increased bone formation especially in the calvarial and clavicles relative to mice$^{Cbfa1+/-}$.

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Neuropathology

Toxicity of lead through free radicals in rat brain

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Studies suggest that some lead-induced toxic effects may occur through free radical production and oxidative stress. This study examined the relationship between brain histopathological alterations and oxidative stress in subchronic lead exposure. Male Albino rats received lead acetate at 0.01%, 0.05% and 0.1% w/v in their drinking water for 30 days. Animals given sodium acetate (0.1% w/v) served as control in the same period. At the end of exposure, blood-lead levels, blood catalase and superoxide dismutase activities and malondialdehyde content (in blood and brain) were measured. The brain tissue samples were prepared and analysed by light and scanning electron microscopy. The results show that, the blood-lead levels in treated animals were higher in comparison with control. Catalase and superoxide dismutase activities in animals treated with 0.01% and 0.05% w/v did not increase in comparison with control ($P > 0.05$) but these values were higher in animals treated with 0.1% w/v lead acetate ($P < 0.01$). Malondialdehyde content in blood and brain of animals treated with lead acetate 0.1% w/v, increased significantly ($P < 0.01$), but these values were not significantly increased in other treated animals. No major histopathological alterations were detected in the brains of animals treated with lead acetate at 0.01% and 0.05% w/v. In animals treated with lead acetate 0.1% w/v, demyelinization and collagenous scar formation with neuronal atrophy in hippocampus region was observed. It is concluded that lead acetate induce oxidative stress which has an important role in brain damage in rats.

Wet SEM: A novel method for rapid diagnosis of brain tumors

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P-23

CK19 mRNA ANALYSIS BY LAMP WITH GD-100 IS APPLICABLE TO AN INTRA-OPERATIVE DETECTION OF MICROMETASTASIS OF LYMPH NODE.

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Introduction; Micrometastasis of lymph node has been detected either by immunohistochemistry or molecular analysis. The former has a difficulty in a quantitative analysis and the latter is time-consuming. The authors applied the CK19 mRNA analysis by loop-mediated isothermal amplification (LAMP) with GD-100 (SYSMEX) to an intra-operative detection of micrometastasis of lymph nodes.
Materials and methods; Five colon cancer cell lines (Lovo, DLD1, WiDr, Colo201, Colo320) and 38 lymph nodes were examined. Cytokeratin (CK) 19 mRNA of cell lines were analyzed quantitatively by LAMP with GD-100 and conventional real-time reverse transcriptase-polymerase chain reaction (RT-PCR). Lymph nodes were examined by histology and LAMP with GD-100.
Results; Cellular CK19 mRNA levels detected with GD-100 of Lovo, DLD1, WiDr and Colo201 varied more widely than those by conventional RT-PCR. Cellular CK19 mRNA of Colo320 could not be detected with GD-100 and was in very low level by real-time RT-PCR. Three of 38 lymph nodes revealed CK19 mRNA of various levels in spite of no detectable carcinoma cells by histological examination, and CK19 mRNA analysis with GD-100 was completed within about 40 minutes.
Conclusion; CK19 mRNA analysis with GD-100 can be applicable to an intra-operative detection of micrometastasis of lymph nodes regardless of some limitations.

P-24

IMMUNOLOGICAL EVALUATION OF SENTINEL NODES IN BREAST CANCER PATIENTS

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Introduction: Recent advances in molecular immunology has enabled us to analyze the antigen-specific immune responses .We have addressed to clarify the immunological status of sentinel nodes (SNs) in breast cancer patients.
Method: Surgical specimens of SNs from 62 primary breast cancer patients, who were preoperatively diagnosed cN0, were analyzed. In SNs and non-SNs, expression of CD83 (mature DCs), IL-12p40, IFN-γ (Th1 cytokine), and IL-4, IL-10 (Th2 cytokine) was evaluated using quantitative real-time PCR on the Light Cycler.
Result: First, CD83 and IFN-γ mRNA expression was compared between SNs and non-SNs. Among 48 patients with negative SN metastasis, expression levels of CD83 and IFN-γ mRNA were significantly lower in SNs than in non-SNs (CD83, IFN-γ, $p<0.05$). Next, the immunological parameters were compared between 48 negative and 14 positive SNs. The positive SNs showed significantly higher expression of CD83, IL-12p40, IFN-γ, and IL-10 mRNA than the negative SNs (CD83, IL-12p40; IFN-γ, IL-10, $p<0.05$).
Conclusion: Cellular immune responses may be less active in SNs than in non-SNs before metastasis. Once metastasis occurred in SNs, specific immune responses, including the DC maturation and Th1 up-regulation, may be triggered and developed.