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Anti-Human T-Lymphotropic Virus Type-I Antibodies in Atomic-Bomb Survivors

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Adult T-cell leukemia (ATL), induced by human T-lymphotropic virus type-I (HTLV-I), is endemic in Nagasaki, Japan. To investigate the effects of atomic-bomb radiation on development of this specific type of leukemia, 6182 individuals in the Radiation Effects Research Foundation (RERF) Adult Health Study sample in Hiroshima and Nagasaki were examined for positive rate of HTLV-I antibody. Several lymphocyte parameters were also studied for 70 antibody-positive subjects in Nagasaki. The HTLV-I antibody-positive rate was higher in Nagasaki (6.36%) than in Hiroshima (0.79%) and significantly increased with increasing age, but no association was observed with radiation dose. Whether relationship existed between antibody titer levels and radiation dose among antibody-positive subjects was not clear. The frequency of abnormal lymphocytes tended to be higher in antibody-positive subjects than in antibody-negative subjects, and higher in females than in males regardless of radiation dose. The lymphocyte count was lower in antibody-positive subjects than in antibody-negative subjects and lower in female than in male subjects. No evidence was found to suggest that atomic-bomb radiation plays an important role in HTLV-I infection.

INTRODUCTION

Adult T-cell leukemia (ATL), frequently observed in southwestem Japan and caused by human T-lymphotropic virus type-I (HTLV-I)¹⁻⁵, is characterized by the tumorous proliferation of CD4-positive (helper/inducer) subsets of T-lymphocytes. The diagnosis is established by monoclonal integration of HTLV-I to DNA. ATL is endemic in Nagasaki and is found in the

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population of atomic-bomb (A-bomb) survivors⁶. In A-bomb survivors, frequencies of chromosome aberrations in T-lymphocytes are related to radiation dose⁷⁻⁸. Regarding the function of lymphocytes in the A-bomb survivors, the response to phytohemagglutinin (PHA) decreases with age more in persons exposed to 2 Gy or over than in controls⁹; the mixed-lymphocyte-culture (MLC) response decreases in proportion to increasing dose in A-bomb survivors 15 years or more old at the time of the bombings (ATB)¹⁰; and T lymphocytes decrease in the peripheral blood of elderly A-bomb survivors¹¹. The effects of radiation on the occurrence of virus-induced leukemia is a subject of great interest, but whether A-bomb radiation plays a role in the course from HTLV-I infection to the occurrence of ATL is unclear. No epidemiologic evidence suggests that the incidence of ATL is increased in proportion to the radiation dose^{12,13}. Epidemiological studies on the relationship between ATL and exposure to A-bomb radiation have been limited by the paucity of ATL cases in the Life Span Study (LSS) sample, which occur exclusively among individuals infected with HTLV-I. Approximately one case of ATL occurs per 1000-2000 person-years at risk among infected individuals. Therefore, in this paper, we investigate the relationship between HTLV-I antibody prevalence and radiation exposure to assess indirectly the influence of radiation on ATL.

To study the effects of A-bomb radiation in HTLV-I infection, we examined the relationship between the positive rate of anti-HTLV-I antibody and radiation dose in the Adult Health Study (AHS) sample in both Hiroshima and Nagasaki. AHS subjects, a subset of the LSS sample, have been receiving biennial health examination since 1957. For antibody-positive subjects, the relationship between radiation dose and several parameters such as HTLV-I antibody titer, T-cell subsets and the variation in the ratio of abnormal lymphocytes were studied.

MATERIALS AND METHODS

In Nagasaki, the study subjects consisted of 3090 persons (1196 males and 1894 females, 39-92 years old) who received regular AHS examinations for approximately two years beginning January 1985. In Hiroshima, 3092 persons (1045 males and 2047 females, 39-97 years old), approximately the same number as that in Nagasaki, were selected from among those who received regular AHS examinations during the same period.

The indirect-fluorescence-antibody method was used to detect anti-HTLV-I antibody. The serum to be tested was diluted 10-fold and placed on the ATL cell-line cells (MT-1 and MT-2) as antigen cells for reaction at 37°C for 30 minutes. Fluorescein isothiocyanate (FITC)-labelled goat antihuman IgG serum was added as the secondary antibody, allowed to react similarly, and examined by fluorescence microscopy to determine whether fluorescent-positive cells can be identified. Antibody titers were measured using multiple dilution serum, beginning at 10-fold dilution. The maximum dilution whereby fluorescent-positive cells are identifiable was determined as the antibody titer.

For antibody-positive cases in Nagasaki, the ratio of mononuclear cells in the peripheral blood that reacted with CD3, CD4, and CD8 was determined by fluorescence microscopy. The

frequency of abnormal lymphocytes and atypical lymphocytes among 100 lymphocytes in peripheral blood was determined morphologically. An abnormal lymphocyte was characterized as the cell with multi-cleaved nuclei and dense chromatin often seen in ATL patients. An atypical lymphocyte was the cell with single indentation usually seen in cases of viral infection.

Our data analyses had four objectives: (1) to determine whether the positive rate (prevalence) of anti-HTLV-I antibody varied with A-bomb-radiation dose, age, sex, or city of exposure; (2) to determine whether the magnitude of response as measured by titer levels among positive responders depended on these factors; (3) to determine whether any of several lymphocyte measures depended on response status (positive or negative), sex, age, or radiation dose.

A straightforward logistic regression analysis was performed to meet objective (1). Multiple regression analysis for analyzing titration data was used to meet objective (2). Logistic regression and multiple regression and multivariate regression analyses were used to meet objectives (3).

RESULTS

1. Positive rate of HTLV-I antibody

Of the above subjects, 887 persons lacking Dosimetry System 1986 (DS86) doses, those 222 persons who were born after the bombings, and 346 persons not in either city at the time of bombings (NIC) were excluded from analysis. Therefore, 4731 subjects were available for statistical analyses, including 200 antibody-positive subjects. Table 1 shows the prevalence of antibody positivity by city and sex. Logistic regression analysis was performed on the relationship between the anti-HTLV-I positive rate (prevalence) and city, sex, age at measurement,

Table 1. HTLV-I Antibody-Positive Rate (adjusted) in the AHS Sample, Hiroshima and Nagasaki

Hiroshima		Nagasaki	
Male	Female	Male	Female
7/835 (0.75%)	22/1795 (0.82%)	62/796 (6.04%)	109/1305 (6.57%)
	29/2630 (0.79%)		171/2101 (6.36%)

Table 2. Logistic Regression Analysis on the HTLV-I Antibody-Positive Rate ($n=4731$)

Variable	Estimate	s.e.	p
Intercept	-4.89	0.243	—
City (N vs H)	2.14	0.205	0.000
Sex (F vs M)	0.0893	0.155	0.563
Age-50	0.0223	0.00656	0.001
BM-dose	0.04857	0.104	0.646

Note: Pearson $X^2=4711.33$; N=Nagasaki; H=Hiroshima; F=female; M=male.

and bone-marrow radiation dose in the 4731 subjects. The probability of antibody positivity can be estimated by the following regression formula using the estimated values in Table 2:

$$\text{Probability of HTLV-I positive for a subject} = \frac{e^{\delta}}{1+e^{\delta}}$$

$\delta = -4.89 + 2.14C + 0.0893S + 0.0223(\text{Age} - 50) + 0.0486 \text{ BM-dose (Gy)}$, where, C indicates city (C=0 for Hiroshima and 1 for Nagasaki), S indicates sex (S=0 for males and 1 for females), Age is age at the time of measurement and BM-dose is bone-marrow radiation dose (Gy). Because more than 40 years had passed since the bombing, Age - 50 was employed as variable.

The positive rate of HTLV-I antibody in Nagasaki was 6.36%, statistically significantly different ($p=0.001$) from the positive rate of 0.79% in Hiroshima. The antibody-positive rate increased with increasing age ($p=0.001$) but showed no difference by sex ($p=0.563$). Figure 1 shows the estimated HTLV-I antibody-positive rate by age at measurement. No significant relationship was observed between the antibody-positive rate and bone-marrow dose ($p=0.646$). Figure 2 shows the estimated HTLV-I antibody-positive rate by dose group. Estimated relative risk of HTLV-I antibody positivity is 8.5 for Nagasaki compared to Hiroshima. An estimated increase in log relative risk was 2% per year of age.

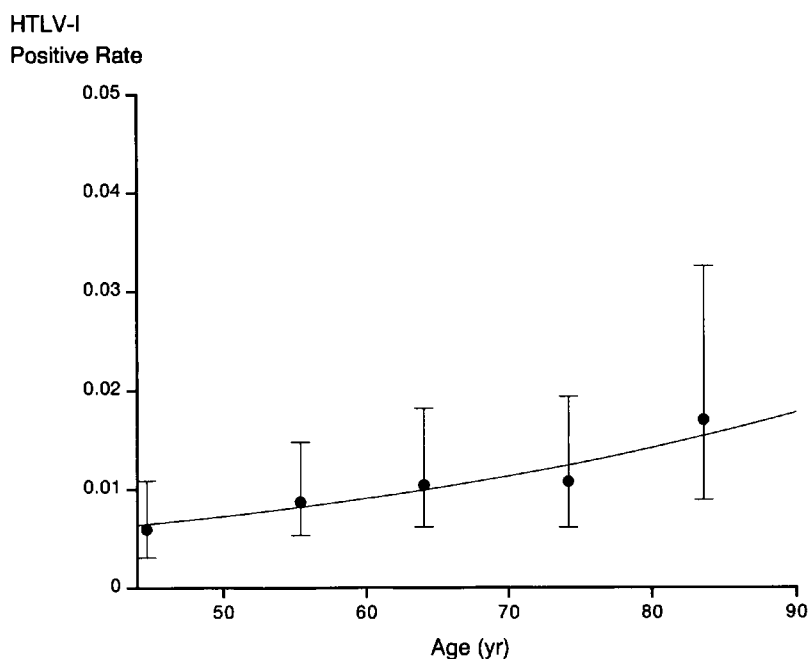


Fig. 1. The Relationship between HTLV-I antibody-positive rate and age. The antibody-positive rate with increasing age ($p=0.001$).

2. Antibody titer

The number of subjects living in the cities ATB with known radiation dose, HTLV-I antibody positivity, and measured antibody titer was 166 excluding those exposed in utero. The

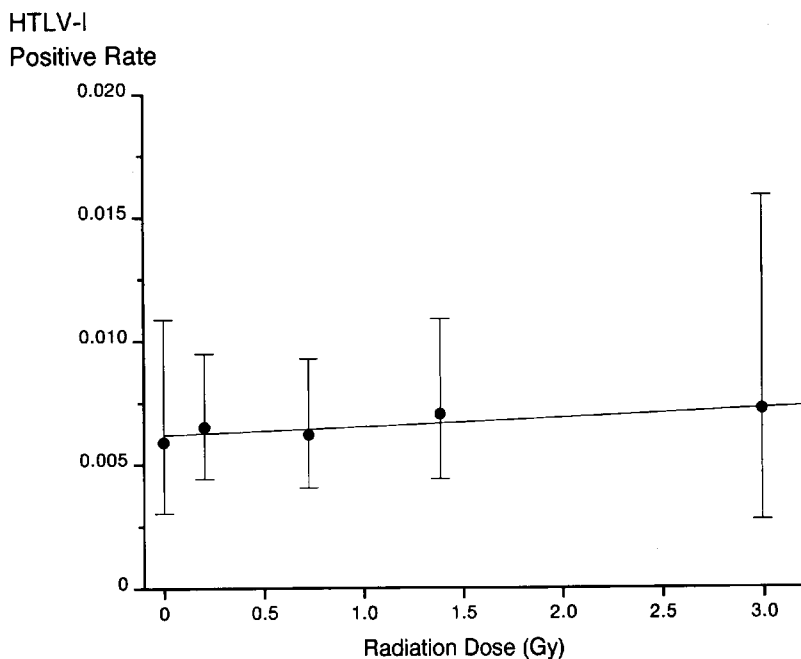


Fig. 2. The Relationship between HTLV-I antibody-positive rate and radiation dose among A-bomb survivors. No significant relationship was observed ($p=0.646$).

Table 3. Regression Analysis on Titer Measurement Values for 166 Subjects

Variable	Estimate	s.e.	p
Intercept	4.391	0.381	—
city	-0.450	0.318	0.158
Sex	0.0822	0.249	0.742
Age-50	-0.0040	0.0101	0.693
BM-dose	0.221	0.164	0.871

relationships among the measured titer values, cities, age at the time of measurement, and bone marrow radiation dose was assessed by multiple regression analysis. Table 3 shows the parameter-estimation values, standard deviations, and p-values. No significant association between any one variable and titer was observed.

3. Lymphocytes in antibody-positive subjects

The number of subjects with available values of various lymphocyte measurements was 83, all Nagasaki A-bomb survivors, including 70 who were HTLV-I antibody positive and 13 who were antibody negative.

3-1 Abnormal lymphocytes and atypical lymphocytes

The frequency of abnormal lymphocytes was 0.73 for antibody-positive subjects and 0.22 for antibody-negative subjects. Table 4 presents the results of logistic regression analysis on the number of morphologically abnormal lymphocytes and the antibody-positive or -negative status of the number of atypical lymphocytes (Ab^+), sex, age at the time of measurement, and bone-marrow radiation dose (BM dose). As shown in Table 4, Ab^+ ($p=0.079$) and sex ($p=0.054$) were suggestive concerning the frequency of abnormal lymphocytes. $Ab^+=1$ is for the antibody-positive subjects, and $Ab^+=0$ is for the antibody-negative subjects.

Table 4. The Results of Logistic Regression Analysis on Morphologically Abnormal Lymphocytes and Atypical Lymphocytes for 83 Subjects

Variable	Abnormal Lymphocytes			Atypical Lymphocytes		
	Estimate	s.e.	p	Estimate	s.e.	p
Intercept	-6.67	0.736	0.000	-3.04	0.254	0.000
Ab^+	1.14	0.650	0.079	-0.0988	0.213	0.643
Age-50	0.0235	0.0159	0.140	-0.00189	0.00874	0.829
Sex	0.696	0.361	0.054	-0.0363	0.167	0.828
BM-dose	-0.181	0.288	0.530	-0.182	0.126	0.148

3-2 White blood cells, lymphocyte percentage and absolute count

White blood count (WBC) was 6043 cell/ μ l in males and 5031 cell/ μ l in females. The lymphocyte count was 2854 cell/ μ l in females.

Regarding WBC and lymphocytes, the counts for females were significantly lower than those for males ($p=0.002$ for WBC, $p=0.010$ for lymphocytes). The lymphocyte percentage for the antibody-positive subjects (44.1%) was significantly lower than that for the antibody-negative subjects (50.5%) ($p=0.032$) (Table 5).

Table 5. Regression Analysis of Titer Measurement Values ($n=83$)

Variable	WBC (x 100)			Lymphocyte count			Lymphocyte percentage (%)		
	Est.	s.e.	p	Est.	s.e.	p	Est.	s.e.	p
Intercept	62.1	5.56	0.000	3194	331	0.000	52.0	3.67	0.000
Ab^+	4.95	4.65	0.291	-123	277	0.658	-6.73	3.07	0.032
Age-50	-0.242	0.188	0.201	-16.4	11.2	0.148	-0.091	0.124	0.463
Sex	-11.3	3.59	0.002	-567	214	0.010	-0.203	2.368	0.932
BM-dose	-2.53	2.24	0.264	-162	134	0.230	-0.647	1.48	0.663
Multivariate test									
Ab^+	$p=0.113,$		Age-50	$p=0.533$					
Sex	$p=0.019,$		BM-dose	$p=0.664$					

Note: WBC=white blood count; est. =estimated

3-3 *Lymphocyte subsets*

The number of CD3 cells decreases with increasing age ($p=0.065$) and is lower in females ($p=0.079$). The number of CD4 cells decreases with increasing age ($p=0.080$) and is significantly lower in females ($p=0.025$). The number of CD8 cells was lower in females ($p=0.033$). A significant association was not observed between these three subsets and BM-dose ($p>0.162$). The subjects may be too few to demonstrate the relationship between T-lymphocyte subsets and dose.

DISCUSSION

There was no statistical correlation between HTLV-I positivity and radiation dose. Since the HTLV-I virus is transmitted primarily from mother to child, radiation would not be expected to be involved in the frequency of virus infection per se. However, virus carriers often reside in clusters in specific regions. A biased distribution of carriers in high- and low- dose regions in Nagasaki might, therefore, influence the relationship between ATL and radiation dose among Nagasaki A-bomb survivors. The results of the present study seem to eliminate this possibility. From the radiation doses in Nagasaki one can infer that no significant bias existed in the distribution of HTLV-I carriers. Thus, this factor can probably be ignored in investigations concerning ATL incidence in the LSS sample.

Interestingly, although the lymphocyte percentage was significantly lower among HTLV-I carriers than among non-HTLV-I carriers, no significant difference was noted in the lymphocyte count, perhaps because of some kind of chronic stimulation exerted on granulocytes by continued HTLV-I infection. Abnormal lymphocytes were frequently observed in HTLV-I carriers, but no association with radiation dose was noted. In addition, the relationship in HTLV-I carriers between the number of T lymphocytes and dose was not clear. Therefore, A-bomb irradiation probably did not play an important role in development of ATL among HTLV-I carriers.

Regarding the immunity of A-bomb survivors to infection, the frequency of hepatitis B surface antigen was higher in highly exposed groups¹⁴. However, no difference was found between two exposure groups in prevalence of antibody for hepatitis B antigen, suggesting the depressed immune competence in heavily irradiated survivors. EB virus antibody titer is higher in high-dose survivors^{15,16}, and the infected virus is assumed to be insufficiently controlled as a result of factors such as the decreased immunological ability of T lymphocytes. However, no association between the HTLV-I antibody titer and radiation dose was noted in this investigation. Therefore, these results suggest dose does not play major role in HTLV-I infection or in the subsequent development of ATL.

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