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Reactivity of the Serum from A-Bomb Survivors with the Tissues of Stomach, Liver and Kidney of Normal Rats

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ABSTRACT

In order to evaluate delayed effects of radiation on pathological immune response an attempt was made to detect antibodies in the serum of atomic bomb survivors against kidney, liver, and parietal cells from rats. The following results were observed.

Analysis of changes in antibody detection frequencies by age and exposure dose without considering sex showed that the rates for those exposed to 100+ rad showed a trend to increase with age for all three organs ($P<0.01$). However, in the 0 rad group, a significant trend to increase with age was noted for anti-kidney and anti-liver antibodies only ($P<0.01$ for both).

Analysis of changes in antibody detection frequencies by sex, age, and exposure dose showed that the detection frequencies increased significantly with age for all three organs in males exposed to 100+ rad ($P<0.05$), but only the anti-liver antibody frequency increased significantly with age in males in the 0 rad exposure group. Females failed to shown any statistical changes in any exposure group.

It has been demonstrated that transitory disorders occur in the immunologic mechanism as acute effects of radiation. There are also a large number of studies suggesting the development of immunologic dysfunction as a late effect of exposure to ionizing radiation. At present, it is unknown to what extent immunologic dysfunction exists in A-bomb survivors in Hiroshima and Nagasaki. If there is a long-term impairment of immunologic competence in A-bomb survivors, the consequences of this dysfunction would be serious from the viewpoint of oncogenesis, aging, resistance to infectious diseases, and development of autommune diseases.

With the aim of detecting late effects of radiation, a number of studies in the field of immunology have been and are being conducted on a study sample in Hiroshima and Nagasaki[1,3-11,16)]. No correlation with radiation exposure has been found for the major erythrocyte antigens, parietal cell antibody, hepatitis-associated antigen and antibody, or serum antibody titer of EB virus antibody[1,5-8)]. Recently, one of the research projects investigating the

Adult Health Study (AHS) sample was expanded to clarify whether the HB antigen titer is excessively high in the group exposed to 100+ rad. The results obtained to date suggest possible radiation effects[9]. Serum immunoglobulin levels (I_gG, I_gA, and I_gM) in A-bomb survivors were found to be within normal limits[11]. A study of the response of serum antibody to Asian influenza vaccine suggested impairmant of antibody synthesis against one of the influenza viruses which was prevalent in Japan around 1945[10].

Several types of antibodies have been found in the AHS sample[1]. However, the total frequency of antibodies in this sample, and its relationship with age, sex, and radiation exposure have remained unclear.

As part of a research project to assess delayed effects of radiation on humoral immunity, an attempt was made to detect various organ-specific antibodies in the serum of A-bomb survivors. The results of the comparative study of the detection frequencies of these antibodies, conducted in relation to sex, age, and radiation exposure dose will be reported here.

MATERIALS AND METHODS

The base population for the antibody immunofluorescent assay consists of all A-bomb survivors exposed to 100 rad or more, who underwent the AHS examination cycle 9 (1974—76) in either city and whose plasma and serum specimens are cryopreserved. Their controls (0 rad group) were matched by age and sex (Table 1).

Immunofluorescence Method

Anti-kidney antibodies, anti-liver antibodies, and anti-parietal cell antibodies were detected by the immunofluorescence method. Kidney, liver, and stomach were removed from a rat and frozen by Cryoquick to form one block of the three organs. Then, the block of three organs was sliced with Cryostat, and mounted on a slide. The prepared tissue sections were placed in a wetting chamber, and one drop each of the undiluted serum was placed on the three-organ specimens and allowed to react at 37°C for 30—45 min, after which the sections were washed for 15 min in a phosphate buffered saline (PBS). After removal of the excess solution, they were again placed in the wetting chamber, and one drop of FITC-labeled antihuman I_gG (antihuman I_gG-FITC) was placed on each organ specimen, and incubated at 37°C for 30—45 min. After washing in PBS, the sections were embedded in glycerin and examained under a fluorescent microscope. Positive control serum obtained from a patient with systemic lupus erythematosus (SLE) was used as the standard of determination in each test.

The nucleus of liver cells dyed diffusely with SLE serum in the present experiment, while fluorescent staining was observed on a part of

Table 1. Composition of Study Population

Age at examination	Dose in rad	Male	Female	Total
<40	0	25	31	56
	100+	17	29	46
	Total	42	60	102
40—49	0	58	93	151
	100+	65	99	164
	Total	123	192	315
50—59	0	31	96	127
	100+	28	94	122
	Total	59	190	249
60—69	0	43	65	108
	100+	52	71	123
	Total	95	136	231
70<	0	42	59	101
	100+	39	52	91
	Total	81	111	192
	Total sample	400	689	1089

the cytoplasm as well as on the nucleus of parietal cells of the stomach and kidney cells. Serum which showed a similar stainability was assumed to be autoantibody-positive.

RESULTS

Antibody detection Frequencies

The increasing trends of the detection frequencies for the antibodies corresponding to each organ are shown in relation to sex and age in (Fig. 1). When single regression analysis was applied to the age-specific detection frequencies for each organ and the trends of the regression coefficients were examined, a trend to increase with age was seen for all three organ antibodies.

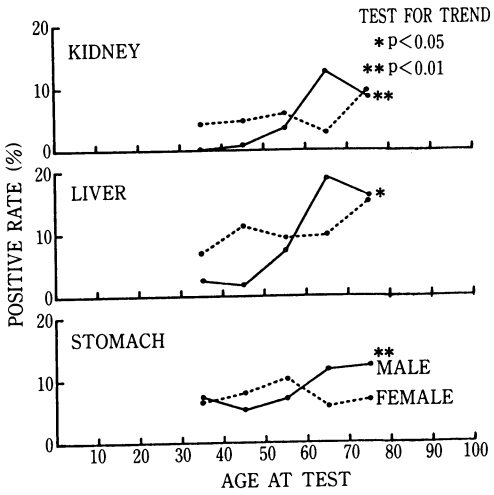

Fig. 1. Trends of detection frequencies for antibodies in serum by sex and age

More particularly, in males this trend was significant for the three organs (kidney and stomach $P<0.01$, liver $P<0.05$).

Antibody Detection Frequencies in Relation to Exposure Dose

Male exposed to 100+ rad showed a higher antibody detection frequency in all three organs, while the reverse was found to hold in females. Females in the 100+ rad group showed a lower rate than the 0 rad group, but there was no significant difference in relation to sex by exposure dose. For sexes combined, the detection frequencies showed no significant difference between the 0 rad group and the 100+ rad group (Table 2).

Antibody Detection Frequencies by Age and Exposure Dose

Since the detection frequencies for antibodies were found to increase with age as shown in Fig. 1, further analysis was conducted in relation to exposure dose and age. As indicated in Fig. 2, the detection frequencies for anti-kidney and anti-liver antibodies increased with age in both the 0 rad and the 100+ rad groups ($P<0.01$). Although antigastric mucosa antibodies failed to shown any definite changes in the detection frequency with age for the 0 rad group ($P>0.1$), in the 100+ rad group there was a significant increase with age ($P<0.01$).

Antibody Detection Frequencies by Sex, Age, and Exposure Dose

The analysis of trends presented in Fig. 3 was reviewed by sex, age and exposure dose. Interestingly, the 100+ rad group of males showed significant increasing trends in antibody detection frequencies with age for all organs

Table 2. Detection Frequencies for Antibodies in Serum by Sex and Exposure Dose

Organ	Dose in rad	Male		Female		Total	
		Number of specimens	Detection frequencies of possible cases	Number of specimens	Detection frequencies of possible cases	Number of specimens	Detection frequencies of possible cases
Kidney	0	196	4.59%	343	7.58%	539	6.49%
	100+	199	6.53	343	4.08	542	4.98
Liver	0	196	9.18	343	11.66	539	10.76
	100+	199	10.05	343	9.91	542	9.96
Stomach	0	196	7.14	343	9.04	539	8.35
	100+	199	10.05	343	7.29	542	8.30

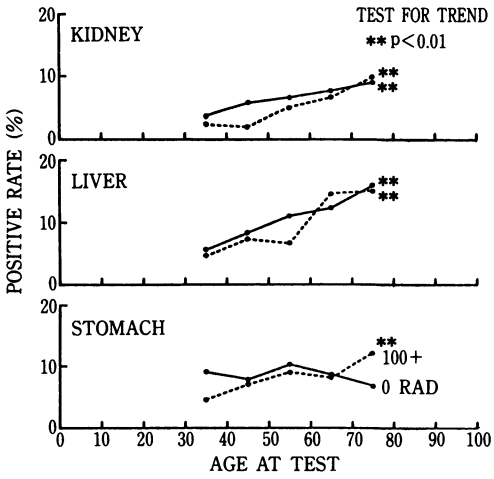

Fig. 2. Trends of detection frequencies for antibodies in serum by age and exposure dose

($P<0.01$). The 0 rad group showed a significant increase only for the liver ($P<0.01$). In females, the increasing trends of antibody detection frequency with age were not statistically significant, despite differences in exposure data.

DISCUSSION

In order to evaluate the late effects of radiation exposure from an immunologic standpoint using serum specimens obtained from A-bomb survivors, the frequency of detecting antibodies was examined in relation to sex, age and exposure dose. As a result, when viewed in relation to sex and age, the antibody detection frequencies showed a trend to increase with age for each organ used (kidney, liver and stomach). This trend was especially clear in the case of males, but not in females. It is conjectured that this difference in the trend of detection frequen-

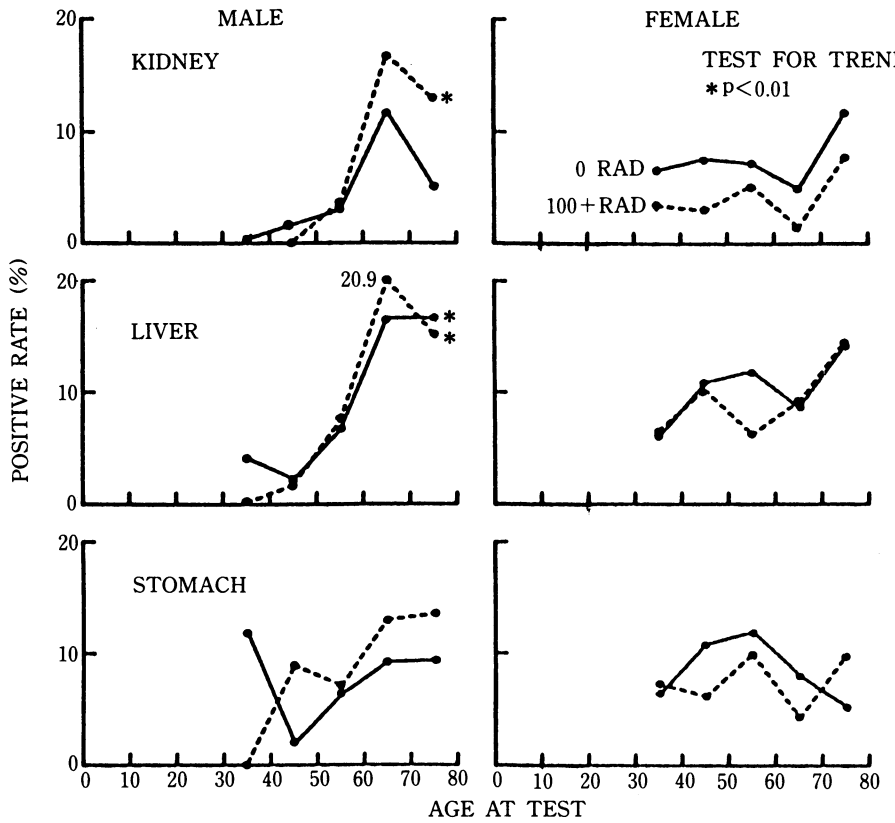

Fig. 3. Trends of detection frequencies for antibodies in serum by sex, age and exposure dose

cies between males and females may reflect certain effects occasioned by pregnancy in female groups.

In order to investigate the effects of A-bomb exposure, it is probably most reasonable to analyze the data on males. Among the male group exposed to 100+ rad, a significant increasing trend in antibody detection frequencies for all three organs has been observed with age, though the 0 rad group also showed a significant increasing trend for the liver.

It has been reported from animal experiments that both young and old animals whose T cell dependent-type immune activity is low are susceptible to the long-term effects of radiation[12-15]. There are few reports dealing with this matter in humans, either at the whole body or cellular level. There is only a report resulting from experiments by the present investigators, that there may be an accelerated decrease with age in cellular immunity in the heavily exposed group (100+ rad) as compared with the 0 rad group[2]. Therefore, at this time, the present findings suggest that there is not enough evidence to shows that a radiation effect on the antibody detection frequencies is associated with acceleration of immunologic aging.

Continuation and extension of such basic research on human immunologic function in relation to radiation exposure is needed. Unless such studies are continued, we will be unable to cope adequately with the acute and chronic immunologic disorders in aging A-bomb survivors.

REFERENCES

1. **Akiyama, M., Yamakido, M., Otake, M., Belsky, J. L., Pastore, J.O., Kawamoto, S., Ohkawa, T. and Dock, D.S.** 1977. Stomach cancer screening in the Adult Health Study population, 1971—1972, Hiroshima. Radiation Effects Research Foundation Technical Report 7—77.
2. **Akiyama, M., Yamakido, M., Kobuke, K., Donal, S.D., Hamilton, H.B., Awa, A.A. and Kato, H.** 1983. Peripheral lymphocyte response to PHA and T cell population among atomic bomb survivors, Radiat. Res. **93**: 572—580.
3. **Barreras, R.F. and Finch, S.C.** 1974. Peripheral blood leukocyte phagocytosis and respiratory response to certain macromolecular substances in the ABCC-JNIH Adult Health Study, Hiroshima, Atomic Bomb Casualty Commission Technical Report 8—74.
4. **Belsky, J.L., Ishimaru, T., Ohashi, T., Robertson, T.L. and Taniguchi, B.** 1972. Leukocyte response to exercise in atomic bomb survivors, Radiat. Res. **50**: 699—707.
5. **Belsky, J.L., King, R.A., Ishimaru, T., Yamakido, M., Kawamoto, S., Hamilton, H.B. and Nakahara, Y.** 1972 Persistence of hepatitis-associated antigen within a fixed population. Arch. environ. Health **25**: 420—425.
6. **Belsky, J.L., Okochi, K., Ishimaru, T. and Hamilton, H.B.** Hepatitis-associated antigen and antibody in A-bomb survivors and nonexposed subjects in Hiroshima and Nagasaki. Radiat. Res. **52**: 528—535.
7. **Hollingsworth, J.W., Hamilton, H.B., Beebe, G.W. and Yamasaki, M.** 1961. Blood group antibody levels in Hiroshima. Blood **17**: 462—473.
8. **Kato, H., Ito, Y., Kawamura, Jr.A., Hamilton, H.B., Belsky, J.L., Nagata, K., Hamajima, K. and Shimizu, Y.** 1976. EB virus infection among A-bomb survivors. Report I. Relation to radiation dose and laboratory findings. RERF Immunology Workshop, p. 3E.1—3E.2.
9. **Kato, H., Mayumi, M., Nishioka, K. and Hamilton, H.B.** 1983. Cross-sectional study on the relationship of HB antigen and antibody and radiation in the adult Health Study sample,1975—77, Am. J. Epidemiol. **117**: 610—620.
10. **Kanamitsu, M., Morita, K., Finch, S.C., Kato, H. and Onishi, S.** 1966. Serologic response of atomic bomb survivors following Asian influenza Vaccination, Jpn. J. Med. Sci. Biol. **19**: 73—84.
11. **King, R.A., Milton, R.C. and Hamilton, H.B.** 1973. Serum immunoglobulin levels in the ABCC-JNIH Adult Health Study, Hiroshima and Nagasaki, Atomic Bomb Casualty Commision Technical Report 14—73.
12. **Perkins, E.H., Peterson, W.J., Gotrtleib, C.F., Halsall, M.K., Cacheiro L.H. and Makinodan, T.** 1975. The late effects of selected immunosupressants on immunocompetence, disease incidence, and mean life span. I. Humoral immune activity, Mech. Ageing Dev. **4**: 231—239.
13. **Peter, C.P., Perkins, E.H., Peterson, W.J., Walburg H.E. and Makinodan, T.** 1975. The late effects of selected immunosuppressants on immunocompetence, disease incidence, and mean life span. III. Disease incidence and life expectancy. Mech. Ageing Dev. **4**: 251—261.
14. **Peterson, W.J., Perkins, E.H. Goodman, S.A., Hori, Y., Halsall, M.K. and Makinodan, T.** 1975. The late effects of selected immunosupressants on immunocompetence, disease incidence, and mean life span. II. Cell mediated immune activity. Mech. Ageing Dev. **4**: 241—249.
15. **Sado, T.** 1979. Late effects of radiation on immune system. A review. Proc 6th Internat Cong Radiation Res. 688—697.
16. **Wood, J.W., Kato, H., Johnson, K.G., Ueda, Y., Russell W.J. and Duff, B.** 1967. Rheumatoid arthritis in Hiroshima and Nagasaki, Japan, Prevalence, incidence, and clinical characteristics, Arthritis. Rheum. **10**: 21—31.