

Research note

Absence of Positive anti-Human Immunodeficiency Virus Antibody Titers in the Japanese Cases with Invasive Amoebiasis

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A distinct increment in the number of cases with amoebiasis has been noted in Japan since 1980, which is supposed to be primarily attributed to sexually-transmitted amoebiasis among biased males (Takeuchi *et al.*, 1987). This seems to be quite comparable to the recent epidemiological aspect of this enteric infection in the western countries, in particular to that of the United States (Markell *et al.*, 1984). On the other hand, it is well known that homosexual intercourse of biased males has been the biggest cause of acquired immunodeficiency syndrome (AIDS) in the western countries. Moreover, AIDS is frequently associated with protozoan parasites such as *Cryptosporidium* spp. and *Giardia lamblia* as well as *Pneumocystis carinii* because of immune suppression. The concomitant infection of *Entamoeba histolytica* and Human immunodeficiency virus (HIV), the causative agent of human amoebiasis and AIDS respectively, is also naturally found (Sargeant, 1987). Fortunately, however, most of the amoebae isolated from biased males in the western countries, a community of the highest risk for HIV infection, were avirulent. This explains why in spite of the presence of a large

number of AIDS cases virtually none of them died of amoebic infection in the western countries. In contrast, since the rate of transmission of virulent strains of *E. histolytica* among biased males is higher in Japan than in the western countries (Takeuchi *et al.*, 1987) and we have had at least one AIDS case who died of amoebiasis in Japan, it seems to be important to study the correlation of positive anti-HIV titers with invasive amoebiasis in Japan and to know whether HIV infection is widespread among the symptomatic cases with amoebiasis including those with sexually-transmitted amoebiasis.

Fifty eight Japanese cases with amoebic liver abscess, 36 with amoebic dysentery or colitis and 40 with ulcerative colitis and other infectious diseases like toxoplasmosis, bacterial colitis and liver abscess were examined in this study. Moreover, 50 serum specimens randomly selected from healthy Japanese adult males were also tested to know the specificity of anti-HIV antibody titer determination. Symptomatic amoebiasis was confirmed by evident clinical signs and symptoms which seemed compatible with amoebiasis, detection of trophozoite and/or cyst of *E. histolytica* through stool examination, endoscopic biopsy and/or liver puncture, and final serologic examination utilizing gel diffusion precipitin test, indirect immunofluorescent antibody test and counterimmunoelectrophoresis. The cases with amoebiasis included 29 subjects with

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positive syphilis serology as examined by *Treponema pallidum* hemagglutination test (TPHA) and non-treponemal antigen tests like Venereal Disease Research Laboratory (VDRL) slide test, which was found to be highly correlated with sexually-transmitted amoebiasis (Markell *et al.*, 1984). Moreover, at least 8 out of the 29 cases with positive syphilis serology were bi- or homosexuals. On the other hand, neither positive cases of syphilis serology nor biased males were found in the subjects free from the amoebic infection. All of these cases were Japanese adult males.

Examination of anti-HIV antibody titers was done utilizing an enzyme-linked immunosorbent assay (ELISA) kit (HTLV-III/LAV/EIA, Abbott Laboratories, Chicago, Illinois, USA obtained through Dainabot Inc., Tokyo, Japan). This was one of the first series of commercially-available kits for anti-HIV antibody titer determination in Japan. Determination of the antibody titers and calculation of the cut-off value were conducted according to the instruction by the manufacturer. The sera which showed higher absorbance than the cut-off value were judged reactive and further processed to calculate the cut-off index, i.e., the absorbance of a test sera/the cut-off value. The cut-off index of more than 1.00 was designated positive. The sera judged positive in this manner were sent to Dr. S. Tamagawa, Tokyo Metropolitan Komagome Hospital, Tokyo, and to Prof. K. Kurimura, School of Medicine, Tottori University, Tottori Pref., and the confirmative examination was done using indirect immunofluorescent antibody test (IF) and Western blotting analysis by their courtesy.

Distribution of the cut-off index calculated from the antibody titers of these specimens was demonstrated in Fig. 1. It seems evident that none of the ELISA positive sera were found in the cases with ulcerative colitis and other infectious diseases (Group 1). On the other hand, Group II, i.e., the cases with amoebic liver abscess, contained 14 subjects with the cut-off index more than 1.00, who were consequently judged positive. Among these 14 specimens, 2 showed an extremely high cut-off

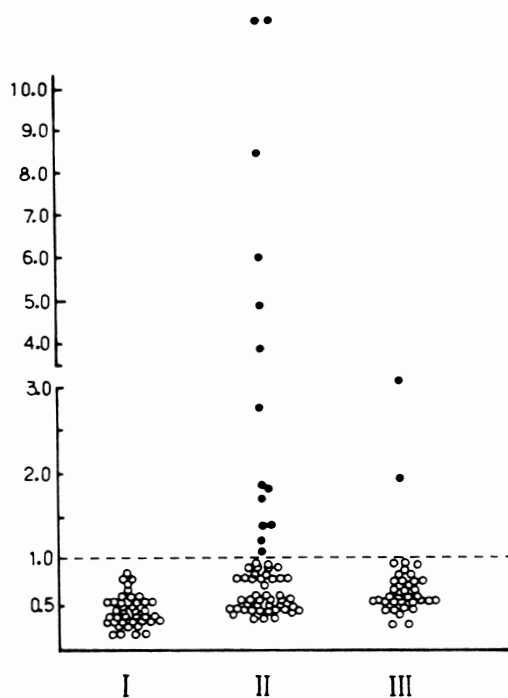


Fig. 1. Distribution of the cut-off index of the Japanese cases with amoebiasis calculated from the anti-HIV antibody titers

Group I: The cases with ulcerative colitis and infectious diseases other than amoebiasis

Group II: With amoebic liver abscess

Group III: With amoebic dysentery or colitis

The vertical line stands for the cut-off index.

index; more than 10.00, and additional 4 more than 3.00. Group III, i.e., amoebic dysentery or colitis, exhibited only 2 cases with the cut-off index in the range of 2.00 to 3.00. The 50 sera from healthy Japanese adult males did not contain any positive subjects. Correlation of anti-HIV antibody titers with syphilis serology and with the sexual history of these cases were also examined in this study; however, no significant correlation could be found between them.

These findings suggest that Japanese cases with amoebic liver abscess had an extremely high rate of positive anti-HIV antibody titers, i.e., 24%. Judging from the data on evaluation of the present ELISA kit with Japanese as the subject (Kobayashi and Yamamoto, 1986;

Kurimura *et al.*, 1986), the sera of the cut-off index more than 3.00 were usually positive by subsequent confirmative IF and Western blotting. At least 6 sera of Group II, therefore, were expected to be positive by the confirmative tests. However, all of the sera judged positive by the present ELISA screening were negative by IF and Western blotting. These observations suggest that HIV infection is not widespread among Japanese cases with amoebiasis including sexually-transmitted amoebiasis. The absence of positive cases by IF and Western blotting seems to be compatible with the lack of correlation of anti-HIV antibody titers with the sexual history and the syphilis serology of these cases. Thus, the epidemiological aspect of HIV infection in Japan appears still different from that of the western countries. The ELISA kit employed in this study may frequently yield a false-positive response to amoebic liver abscess. The reason is not known at present, because such factors as inappropriate storage conditions, repeated freeze-thawing and extensive hemolysis of the specimens, all of which were reported to be responsible for the false-positive reaction of the present ELISA kit (Kurimura *et al.*, 1986), were ruled out. Moreover, these specimens were centrifuged to remove turbidity, if any, before use. Subsequent series of the commercially-

available ELISA kits for anti-HIV antibody titer determination, however, appear to have been improved, since later examinations of some of these specimens with other ELISA kits did not show such a false-positive response.

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