Cross-Sectional Study of Reverse Transcriptase-Inhibiting Antibody as a Marker of Acquired Immune Deficiency Syndrome

MADHU ADVANI,^{1†} DAVID T. IMAGAWA,^{1*} MOON H. LEE,¹ KOUICHI SANO,¹ FATIMA MORALES,¹
RONALD T. MITSUYASU,² AND ROGER DETELS³

Department of Pediatrics, Harbor-UCLA Medical Center, Torrance, California 90509,¹ and Department of Medicine, School of Medicine,² and Division of Epidemiology, School of Public Health,³ University of California, Los Angeles, California 90024

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A cross-sectional study of 128 individuals infected with human immunodeficiency virus type 1 (HIV-1) was conducted to determine the correlation of reverse transcriptase-inhibiting (RTI) antibody to clinical disease. Thirty-two individuals were studied in each of four clinical groups: asymptomatic individuals, those with persistent generalized lymphadenopathy, those with acquired immune deficiency syndrome (AIDS)-related complex, and those with AIDS. Our study showed that 78% of asymptomatic individuals, 53% of those with persistent generalized lymphadenopathy, 50% of those with AIDS-related complex, and only 25% of those with AIDS have RTI antibody. Concurrent measurement of measles antibody level was used as an indicator of the immune status of these individuals. Measles antibody did not decline in persons with clinical disease, but asymptomatic individuals had lower antibody titers, possibly due to hypergammaglobulinemia associated with advanced HIV infection. These results indicate that more HIV-infected asymptomatic individuals than symptomatic individuals have RTI antibody. This suggests either that the RTI antibody level decreases with the progression of disease in HIV infection or that symptomatic individuals do not produce RTI antibody. The presence or absence of RTI antibody can thus be used as a marker of advanced disease.

Reverse transcriptase (RT) is an enzyme residing in the core of the human immunodeficiency virus (HIV) and is essential for viral replication (11, 12). RT is composed of two proteins (p66/p51), and antibody to these proteins is present in approximately 80 to 98% of HIV-infected individuals (1, 3, 10, 13). An antibody that inhibits RT enzyme activity is also found in most HIV-infected asymptomatic individuals and appears not to be directly related to the presence of antibodies against p66/p51, since not all individuals with the p66/p51 antibodies have RT-inhibiting (RTI) antibody (10). There is much interest in the RTI antibody, since it has been shown previously, with a small number of individuals, to be absent during disease development in HIV infection (2, 7). In contrast, studies of p66/p51 antibody showed that it did not vary in different clinical stages of the disease (3).

The production of RTI antibody is not common. There have been reports of only a few other naturally occurring animal retrovirus infections with this antibody: G-murine leukemia virus in AKR mice (4), natural bovine leukemia virus infection in cattle (14), and natural exposure of cats to feline leukemia virus (5). The production of RTI antibody to feline leukemia virus was associated with less detectable virus, and absence of the antibody was accompanied by viremia in cats (5). Similar results were observed with HIV infection in humans (10).

We studied 128 HIV-infected individuals to examine the correlation of RTI antibody to clinical status and to determine the possible use of this antibody as a marker for advanced HIV infection.

MATERIALS AND METHODS

Study subjects. Serum samples for this study were obtained from the UCLA AIDS Clinical Research Center and from the Multicenter AIDS Cohort Study. A cross-sectional study of 128 men was conducted in which 32 homosexual men infected with HIV were randomly chosen from four clinical groups. Centers for Disease Control (CDC) criteria were used to classify the groups as asymptomatic, having persistent generalized lymphadenopathy (PGL) (CDC group II), having acquired immune deficiency syndrome (AIDS)-related complex (ARC) (CDC group IVa), or having AIDS (CDC group IVc). The ages of these men ranged from 21 to 50 years, with a mean age of 36 years. All samples were coded and tested blindly.

IgG purification. Purification of immunoglobulin G (IgG) from sera was achieved by the method of Johnson and Libby (6) with QAE-Sephadex A-50 (Pharmacia) anion exchange chromatography. Diluting solution was prepared by adding 2.88 g of ethylenediamine and 4.38 g of glacial acetic acid to 1 liter of deionized water (pH 7.0). A QAE-Sephadex suspension was made by adding 1 g of QAE-Sephadex powder to 20 ml of the diluting solution and allowing it to swell overnight. Columns were prepared by adding 400 μ l of QAE-Sephadex suspension to disposable minicolumns containing Dacron fiber at their bases as support. The columns were washed twice with diluting solution. Serum (100 μ l) was added to 1.9 ml of diluting solution, layered onto the columns, and left for 15 min at room temperature. The first eluate (2 ml) containing IgG was collected and frozen at -20°C until used for testing.

RTI assay. The RTI assay was performed by the method of Sano et al. (10) with the following modifications. A 4- μ l portion of purified IgG (approximately 1.7 μ g) was mixed with 40 μ l of a stock human T-cell lymphotropic virus type III RT preparation and allowed to react at 4°C for 15 min. Triplicate samples (10 μ l) were then assayed for RT activity

* Corresponding author.

† Present address: Department of Pediatrics, Division of Immunology, University of Maryland School of Medicine, Baltimore, MD 21201.

TABLE 1. RTI antibody in HIV-infected individuals

Clinical status (CDC group)	No. of individuals (%) ^a	
	RTI positive	RTI negative
Asymptomatic	25 (78)	7 (22)
PGL (III)	17 (53)	15 (47)
ARC (IVa)	16 (50)	16 (50)
AIDS (IVc)	8 (25)	24 (75)

^a Statistical analyses were as follows: Pearson chi-square test for overall difference among groups, $P < 0.0001$; test for linear trend, $P < 0.0001$; pairwise comparison, $P = 0.01$ between asymptomatic and AIDS groups only.

at 37°C for 18 to 22 h as described by Lee et al. (8). The amount of [³H]dTMP incorporated into DNA product was counted in a liquid scintillation spectrometer, and the percent inhibition of RT was calculated. Positive inhibition of RT is defined as a $\geq 50\%$ decrease in enzyme activity compared with the activity of control serum of a seronegative individual. For each RTI assay, a known RTI-positive serum and a known RTI-negative serum served as controls.

Measles antibody titer. Immune status of each individual was also evaluated by measuring measles antibody levels. Purified IgG samples were diluted twofold (starting with a dilution of 1:80) and tested. Measles antibody was determined by using commercially available enzyme-linked immunosorbent assay kits (M. A. Bioproducts). Instructions provided with the kits were followed.

Statistical analysis. The results of the RTI antibody and the measles antibody level assays in the four groups were compared by the Pearson chi-square test.

RESULTS

The presence of RTI antibody in the sera of 32 individuals in each of the four clinical groups is shown in Table 1. In the asymptomatic group, 25 (78%) persons had positive enzyme inhibition. In the AIDS group, only 8 (25%) had positive inhibition. Statistical analysis showed a highly significant ($P < 0.0001$) overall difference among the four groups, and the test for linear trend (asymptomatic to AIDS) also showed a highly significant ($P < 0.0001$) difference. Pairwise contrast showed a significant difference only between the asymptomatic and the AIDS groups ($P = 0.01$). We have previously shown that HIV seronegative individuals do not show any RTI antibody activity (10). Three representative RTI antibody dilution curves are shown in Fig. 1 to demonstrate antibody specificity. These curves delineate an inverse relationship between percent inhibition and serum dilution. The geometric mean measles antibody titers of the 32 individuals studied in each of the four clinical groups are as follows: asymptomatic group, 51; PGL group, 226; ARC group, 121; and AIDS group, 161. These titers are expressed as the mean anti-log₂ of the highest serum dilution with antibody. For the calculation of the geometric mean, a titer of $<1:80$ was assigned a value of 40. The Kruskal-Wallis test showed overall differences among groups. The levels of measles antibody did not decrease in the AIDS group. Posttest Helmert contrasts showed that asymptomatic individuals had significantly lower measles antibody titers than individuals with PGL, ARC, or AIDS ($P < 0.0001$). There were no other significant differences.

DISCUSSION

A significant lack of RTI antibody in groups with advancing HIV disease suggests an association between the ab-

FIG. 1. Three representative RTI serum IgG dilution curves for three HIV-seropositive men. Results are expressed as percent inhibition of RT activity.

sence of RTI antibody and the development of AIDS. This study confirms similar findings reported earlier (2, 7). These are interesting observations, since it has been reported that levels of RT (p66/p51) antibody do not correlate with clinical disease (3). The present study further substantiates that p66/p51 antibody does not directly measure the RTI antibody. Sano et al. (10) have compared the Western blots (immunoblots) of individuals producing RTI with the Western blots of those lacking RTI antibody. They found a wide variation in p66/p51 antibody levels between the two groups, with no correlation to RTI. The limitation of our study, which looked at a cross-sectional pattern of RTI antibody among different groups, is that antibody response may vary from person to person. It would be important to monitor the same individual in series from the time of seroconversion to the development of PGL, ARC, or AIDS and to evaluate the RTI antibody pattern of the individual.

Our findings in the present study show no statistical difference between RTI antibody in the ARC and PGL groups. This could be explained by the fact that there is considerable overlapping in the clinical picture of these two groups. Similar findings were noted by Chatterjee et al. (2).

We feel that the decreased presence of RTI with advancing clinical disease is not mere loss of antibody due to an increasing viral antigen load. Data from ongoing longitudinal studies in our laboratory show that some individuals make RTI antibody when they seroconvert, while others do not. Individuals lacking RTI antibody are the ones who, in significantly larger numbers, go on to develop AIDS (M. Advani et al., manuscript in preparation). Others may produce RTI antibody in small amounts, lose it, and subsequently develop AIDS. RTI antibody titers in infected asymptomatic individuals have ranged from $<1:20$ to $1:20,480$ (unpublished data). This raises the question of whether high RTI antibody may be required for inhibiting viral replication. This is suggested by our previous findings,

which showed that individuals with RTI antibody had less viremia than those lacking antibody (10). Similar results were also seen in cats infected with feline leukemia virus (5). Further proof of this concept might be worked out in an in vitro model.

The importance of RTI antibody appears to be twofold. First, the presence of the RTI antibody could be used as a prognostic indicator, since studies have shown that its absence is associated with clinical AIDS. This can be further substantiated by longitudinal studies (manuscript in preparation). Secondly, RTI antibody may be a protective antibody. This is an attractive idea, because RTI antibody may then be useful in the treatment of or in the design of a vaccine against HIV. RTI antibody of infected individuals might be boosted in an effort to suppress the progression of the disease, a concept originally proposed by Salk (9).

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