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Antibody That Inhibits Human Immunodeficiency Virus Reverse Transcriptase and Association with Inability To Isolate Virus

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Most individuals infected with human immunodeficiency virus (HIV), the causative agent of acquired immune deficiency syndrome, produce an antibody against the viral reverse transcriptase (RT). Our studies show that 67% of HIV-seropositive individuals (33 of 49) produced an antibody that specifically inhibited viral RT enzyme activity. We were able to isolate HIV from only 18% of these individuals (6 of 33). On the other hand, virus was readily isolated from 63% of HIV-seropositive individuals (10 of 16) who did not demonstrate this antibody. Further examination of this RT-inhibiting antibody and its role during virus infection is needed, as it may prove to be of diagnostic, prognostic, or therapeutic value in the study and treatment of acquired immune deficiency syndrome.

Human immunodeficiency virus (HIV), the etiological agent of acquired immune deficiency syndrome (AIDS), is a retrovirus and contains a reverse transcriptase (RT) in its virion for making a DNA copy of its RNA genome during replication (1, 4, 9). An RT assay has been used for detecting the retroviruses (15, 16), and since each virus RT is antigenically distinct, the assay has also been used for examining virus relatedness by a specific antibody that inhibits the action of the enzyme (5, 13, 14). This inhibiting antibody has been shown to be reactive against the viral RT and can be prepared in experimental animals by immunizing them with purified viral polymerase (12). The RT of HIV has been reported to consist of two protein species of 66 and 51 kilodaltons (3). The viral polymerase protein appears to be highly immunogenic in humans, with 80% of infected persons producing antibody to it. This is in contrast to most other animal retrovirus infections, in which antibody against the viral RT protein has not been demonstrated (3). It is not known what role, if any, this antibody may play during infection. We therefore studied an RT-inhibiting (RTI) antibody in homosexual male volunteers being followed in the Multicenter AIDS Cohort Study. We report here our findings after examination of 86 men.

The kinetics of RT inhibition by antibody was examined with plasma (heat inactivated, defibrinated, and treated as serum) of a homosexual male that showed high anti-HIV antibody levels. Immunoglobulin (10 μl), which was partially purified from the plasma by two 40% ammonium sulfate precipitations, was mixed with 10 μl of a detergent-disrupted HIV isolate (human T-cell lymphotropic virus type III [HTLV-III]) RT preparation at 4°C from 0 to 90 min and then immediately tested by a standard RT assay procedure (4). Maximum reduction of RT activity by immune serum was seen after a 30-min incubation, and this time period was used for all subsequent experiments. The RT inhibition was dependent upon antibody concentration and decreased with dilution of the immune serum. A control serum from an HIV-seronegative blood donor showed no inhibition of enzyme activity.

To assure that the enzyme-inhibiting factor of the serum was due to immunoglobulin G (IgG) antibody specifically, the partially purified immunoglobulin was passed through a QAE-Sephadex (Pharmacia) anion-exchange column. The void volume contained purified IgG that inhibited viral RT activity as before. Sera from 13 randomly selected individuals were examined by the Western blot (immunoblot) technique (11) for specific antibodies against HTLV-III proteins. These individuals showed a wide variation in the levels of antibodies reactive against the viral proteins (Table 1), making evaluation and comparison difficult. On the whole, however, the 10 individuals with RTI antibody had higher levels of total HIV antibody than did the 3 individuals who did not have the inhibiting antibody. Although the individuals without RTI antibody exhibited a substantially lower level of total HIV antibody, they still retained antibodies against the major viral envelope (gp120), RT (p64/p53), and core (p24) proteins. However, they did show a lower percent level of RT (p64/p53) antibody. It should be noted that one may have a low percent level of p64/p53 antibody and yet still have RTI antibody (Table 1, group A, samples 1 and 6).

We also determined that HIV antibody specifically inhibited the polymerase activity of HTLV-III and not that of other unrelated retroviruses. Four retroviruses were tested and shown to have RT by their greater activity on a synthetic poly(rA) · oligo(dT) template primer, whereas *Escherichia coli* DNA-dependent DNA polymerase was more active on a poly(dA) · oligo(dT) template. The HIV immune serum inhibited RT of two similar HIV isolates, HTLV-III and lymphadenopathy-associated virus (1), but not that of two other retroviruses, HTLV-I and avian myeloblastosis virus, or that of DNA polymerase from *E. coli*.

The results of our tests for HIV antibody and for the presence of virus in 86 individuals are shown in Table 2. A total of 49 persons were seropositive for HIV; 33 of these persons also demonstrated an antibody that inhibited virus RT. Virus isolation was accomplished from the lymphocyte cultures of 6 of the 33 persons having an RTI antibody in their serum and from 10 of 16 persons who did not have the RTI antibody. The difference in the virus isolation rate of

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TABLE 1. Quantitation of serum antibodies to HTLV-III proteins determined by Western blot analysis

Sample[a]	Antibody to HTLV-III protein (% of total antibody):				Antibody to all HTLV-III proteins (relative U)[b]
	gp120	p64	p53	p24	
Standard anti-HIV pool	0.3	6.6	10.4	28.4	100
Group A					
1	0.1	2.6	3.9	44.7	58.7
2	1.0	16.0	16.2	5.8	14.3
3	0.7	4.9	31.5	37.8	106.4
4	0.3	3.2	37.5	34.2	101.5
5	0	10.2	4.8	6.9	10.4
6	20.4	5.0	6.2	19.9	2.7
7	0.4	7.0	2.7	35.6	16.3
8	0.5	23.0	7.0	16.7	14.8
9	0.3	2.1	29.8	46.2	136.4
10	0.2	6.6	34.9	40.2	37.2
Mean ± SD	2.4 ± 6.3	8.1 ± 6.7	17.5 ± 14.4	28.8 ± 15.2	49.9 ± 48.3
Group B					
1	8.1	3.1	5.6	19.1	3.7
2	0	1.2	4.5	59.5	12.9
3	1.3	1.3	0	42.4	9.5
Mean ± SD	3.1 ± 4.4	1.9 ± 1.1	3.4 ± 3.0	40.3 ± 20.3	8.7 ± 4.7

[a] Groups A and B are individuals with and without RTI antibody, respectively.

[b] The percentage of antibody to HTLV-III proteins was related to a standard HIV antiserum pool from 15 homosexual men. This was assigned 100 relative units. The quantitations were done by measurements of intensities of bands on each strip by using a densitometer with a computer-assisted program identifying each viral protein band by relative migration distance and intensity.

these two groups was highly significant ($P < 0.01$). The clinical status of the men studied was unknown at the time of virus testing, and this was evaluated subsequently. The majority of the seropositive men had an inverted T helper/suppressor cell ratio and some presented with generalized lymphadenopathy. AIDS-related complex was diagnosed in 12% (4 of 33) of patients with RTI and 31% (5 of 16) of patients with no RTI. A significant difference was that two cases of AIDS were found in the group lacking RTI antibody but none was found in the group with the antibody. A total of 33 homosexual males and 4 blood donors were HIV seronegative and demonstrated no RTI antibody in their serum, and no virus was isolated from them.

This study confirms the previous report that the majority of HIV-infected persons produce antibody against virus RT (3). Some individuals also produce a polymerase antibody that inhibits enzyme activity. Since this inhibiting antibody is not present in all p64/p53-seropositive individuals, its epitope(s) on the polymerase molecule must be unique from the other RT epitopes. This is shown by the three persons who lacked RTI antibody yet still showed antibodies reactive to other p64/p53 epitopes. It is not known why some individuals have this RTI antibody while others do not. From the present study, we cannot determine whether this RTI antibody reflects a host or virus function or both, nor can we distinguish between either the nonproduction or the loss of antibody during the course of infection. We are currently following these same and other individuals in the Multicenter AIDS Cohort Study to answer these questions.

TABLE 2. Association between RTI antibody and HIV isolation

Sample	No. in group	No. HIV seropositive[a]	No. RTI antibody seropositive[b]	No. with isolatable HIV (%)[c]
Homosexual men	33	33	33	6 (18)[d]
	16	16	0	10 (63)[d]
	33	0	0	0
Blood donors	4	0	0	0

[a] Determined by an enzyme-linked immunosorbent assay and Western blot analysis.

[b] Determined by using partially purified immunoglobulin from plasma, incubation with solubilized HTLV-III, and assay for RT activity (≥25% reduction of the RT activity compared with that in control serum was considered positive inhibition).

[c] Peripheral blood lymphocytes were cultured for 21 days, and the presence of virus was determined by RT assay and antigen capture enzyme-linked immunosorbent assay.

[d] $P < 0.01$.

HIV infection appears to be distinct in the natural production of an inhibitory antibody against the RT, since this antibody has been described in only a few animal retrovirus infections, i.e., AKR mice infected with G-murine leukemia virus (6), cows naturally infected with bovine leukemia virus (17), and cats naturally exposed to feline leukemia virus (7). It is not known how or why this RTI antibody is made in these retrovirus infections. However, these must be unique events of viral polymerase-host immune response interaction that have not been shown to occur in most other retrovirus infections. It is interesting that our findings are similar to those reported for feline leukemia virus. Both viruses cause an AIDS type of disease and produce an antibody that inhibits RT, and the presence of this antibody is associated with the inability to isolate virus (7, 10).

Our findings are in agreement with a recent report on the blocking of HIV RT activity by IgG from seropositive asymptomatic individuals (8). This study also showed the possible relationship between loss of RTI antibody in 4 of 10 individuals and their progression to AIDS-related complex or AIDS. Since progression of HIV infection to AIDS-related complex or AIDS is accompanied by the increased presence of virus or virus-infected cells, this reported clinical finding may correspond with our ability to more readily isolate virus from individuals who lack an RTI antibody. In our study group, we are now examining the relationship of RTI antibody to clinical status to confirm these possibilities.

We postulate two possible explanations for the association

between this RTI antibody and virus isolation. (i) The antibody may play a direct role in moderating the replication of virus in infected persons. At present, there is no direct evidence that this occurs, and if it does, it would probably require the antibody to penetrate an infected cell. RTI may be a possibility, since it has been reported that IgG is taken up and internalized by active lymphocytes (2). RTI antibody probably does not eliminate the virus completely, since we have isolated virus from individuals with this antibody. More likely, the antibody reduces the virus to a low level that is not as readily detected by our present culture testing procedure (4). Further studies are now being done to see if this antibody directly hinders virus replication in vitro. If this proves to be true, then it may have possible therapeutic application in the treatment of infected persons or in the design of a vaccine for the prevention of infection. (ii) The antibody may have only an indirect association with virus isolation, and the presence of the antibody may simply be a reflection of events occurring during infection. These events may be, at one time or another, periods of small and large amounts of virus with corresponding high and low levels of total HIV antibody, or the loss of RTI antibody may be an early indicator of the progression of the host to a more immunosuppressed stage of virus infection. Thus, this antibody may indicate the current state of HIV infection and may have diagnostic or prognostic value.

Further studies are needed to understand the role of this antibody during virus infection.

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ADDENDUM IN PROOF

A more sensitive virus culture procedure has not shown any difference for the isolation of HIV in individuals with or without RTI antibody. Thus, we believe that the association reported here is between the presence of RTI antibody with a lower level of HIV and not the complete absence of virus.

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