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Characterization and Clinical Association of Antibody Inhibitory to HIV Reverse Transcriptase Activity

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Reverse transcriptase activity of the human immunodeficiency virus (HIV) was blocked in vitro by immunoglobulin G (IgG) derived from certain individuals infected with this retrovirus. A heterogeneous immune response for inhibition of enzyme function was noted. Catalytic activity was depressed by 50% or more with the use of 10 micrograms of IgG from 11 of 16 HIV-seropositive asymptomatic carriers, but from 0 of 8 seronegative controls and 2 of 12 patients with acquired immune deficiency syndrome (AIDS) or the AIDS-related complex (ARC). The inhibitor was confined to the F(ab')$_2$ fragment. It was not directed against the poly(rA) · oligo(dT) template, nor against major envelope or structural viral antigens, and did not cross-react with bacterial, avian, or other mammalian DNA polymerases. It did not correlate with recognition of polymerase antigens by radioimmunoprecipitation. Loss of this inhibitor may be associated with development of clinical disease. Ten asymptomatic HIV-seropositive carriers with high titers of IgG antibodies to reverse transcriptase were followed for a mean of 3 years. All of four lost inhibitory capability prior to development of AIDS or ARC, while titers persist in the six who remain clinically healthy.

THE SERUM OF MANY ANIMALS NATURALLY infected with retroviruses contain antibodies capable of blocking the enzymatic activity of particulate reverse transcriptases (*1–4*). In some instances, reactivity occurs in the absence of detectable antibody to viral envelope or structural proteins (*2*). Correlations of antibody to reverse transcriptase (RT) with clinical status have been studied in cattle and cats. Bovine leukemia viral polymerase could be inhibited specifically by antibody from the serum of leukemic cattle, but not from the serum of infected animals experiencing solely a leukocytosis (*2*). In contrast, those cats that were exposed to feline leukemia virus (FeLV) and remained nonviremic or in a low state of viremia had antibody to FeLV polymerase (*1*). No such reactivity occurred in viremic or diseased animals.

Antibodies to the RT of human immunodeficiency virus (HIV), the etiologic agent of the acquired immune deficiency syndrome (AIDS), are readily detectable in the majority of HIV-seropositive individuals (*5–7*). These immunoglobulins, directed against proteins associated with endonuclease and RNA-dependent DNA polymerase functions, can be recognized by immunoblotting and radioimmunoprecipitation techniques and occur independently of the individual's clinical status (*6, 7*). In addition, sera from asymptomatic West Africans infected with the human T-lymphotropic virus type IV (HTLV-IV), a closely related retrovirus, cross-react with HIV polymerase antigens (*7*). Such marked immunogenicity of a polymerase product has not been demonstrated for other mammalian retroviruses, including HTLV-I, in their respective hosts. The significance of these responses is unknown. Some animals infected with retroviruses generate antibody that binds to RT without neutralizing it (*8*).

In one approach to this problem we obtained serum samples from individuals with evidence of exposure to HIV as determined by immunoblotting for antigens in lysates of purified HIV. Immunoglobulin G (IgG) was isolated from each serum by ammonium sulfate fractionation and DEAE-cellulose column chromatography (*9*) or Zeta-Chrom 60 filter separation (AMF Lab Products). F(ab')$_2$ fragments of selected IgGs were prepared by digestion of the IgG with pepsin followed by chromatographic purification (*10*). HIV virions were obtained from culture supernatants of chronically infected H9 CD4$^+$ lymphoblasts (*11*). Virus recovered from clarified supernatants precipitated with polyethylene glycol 4000 were solubilized in 0.8*M* NaCl, 0.5% Triton X-100, 0.5 m*M* phenylmethylsulfonyl fluoride (PMSF), 50 m*M* tris (*p*H 7.9), 1 m*M* dithiothreitol, and 20% glycerol. Neutralization of RT activity was performed by incubating IgG or IgG fragment with disrupted HIV for 4 hours at 4°C. Enzyme assays were based on the method of Goodman and Spiegelman (*12*), as modified for the cation and template preferences of HIV (*13*). None of the IgG samples tested had measurable RT activity in the absence of added HIV polymerase.

As shown in Fig. 1, incorporation of [^{3}H]deoxythymidine monophosphate (dTMP) into DNA by the viral polymerase was blocked by IgG obtained from certain HIV-seropositive individuals. Using a screening dose of 10 μg of whole IgG per reaction mixture, we found that 11 of 16 asymptomatic viral carriers inhibited RT activity by ≥50%. In contrast, 0 of the 8 samples from seronegative controls and 2 of 12 samples from patients with AIDS or AIDS-related complex (ARC) were suppressive at this concentration. The seronegative controls included six male laboratory workers, one female with HTLV-I–associated CD4$^+$ lymphoma, and one female who gave a "false positive" reaction for antibody to disrupted HIV by enzyme-linked immunosorbent assay (ELISA). The erroneous reaction in this pregnant woman was confirmed by negative ELISA competition for viral antigen. The seropositive groups were all homosexual males, age-matched to the control group. The symptomatic group included six AIDS patients (four with *Pneu-*

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mocystis carinii pneumonia and two with Kaposi's sarcoma) and six ARC patients. Both individuals with RT antibody titers >50% had Kaposi's sarcoma as their sole clinical manifestation of AIDS.

Dose-response curves for three representative HIV-seropositive carriers and two controls are illustrated in Fig. 2. The lack of cross-reaction with IgG from an individual with HTLV-I–associated malignancy is apparent. A rabbit IgG prepared against disrupted HIV abrogated RT function (Fig. 2), while a rabbit polyclonal antibody to the envelope antigen gp41 had no effect.

Five seropositive IgG samples that did not suppress RT activity at the 10-μg screening dose were randomly chosen for further analysis. No specific inhibitory capacity was seen at concentrations up to 150 μg of IgG per reaction mixture. Some IgGs showed a slight and variable enhancement of HIV-associated RT activity (Figs. 1 and 2). This phenomenon has been demonstrated in other mammalian retroviral systems with normal sera (*2*), and attributed to protection of the enzyme from thermal inactivation during prolonged incubations.

The time course for inhibition was evaluated by treatment of RT with IgG for various periods at 4°C and then adding synthetic template. In the absence of inhibitor, RT function decreased minimally upon extended incubation (Fig. 3). In contrast, the slope of the curve with an inhibitory IgG was steep, with >50% of the total suppression of catalytic activity occurring within 30 minutes. This experiment was repeated with the same concentration of $F(ab')_2$ fragments of an inhibitory IgG. Similar results were obtained.

The site of IgG action was examined by assaying for RT in the presence of increasing amounts of either solubilized virus or poly(rA) · oligo(dT). Inhibition was diminished by raising the concentration of RT associated with disrupted HIV in the final reaction mixture, whereas altering the template concentration had no effect (Fig. 4). These observations, coupled with the requirement for incubation of the polymerase with IgG for functional inactivation, further suggest enzyme binding as the most likely mechanism of action.

Polymerases of different mammalian retroviruses possess distinct as well as cross-reactive antigenic determinants. This was shown by inhibition studies with antisera derived from infected rodents or immunized rabbits (*14*). The specificity of HIV-associated IgGs for other polymerases was therefore examined. IgG from an individual with a high titer of anti-HIV RT activity did not affect polymerase function of avian myeloblastosis, Moloney murine leukemia, or FeLV viruses (Table 1). It also did not block *Escherichia coli* DNA polymerase type I activity with poly(rA) · oligo(dT) or poly(dA) · oligo(dT) templates. Since these enzymes were all assayed in the presence of poly(rA) · oligo(dT), the results further indicate that inhibition is not related to template inactivation. Only the rabbit IgG prepared against disrupted HIV showed some cross-reactivity with FeLV polymerase at high concentrations.

Tests for nuclease activity, in which inhibitory and control IgGs were incubated with [^{3}H]thymidine-labeled DNA, did not lead to loss of acid-precipitable radioactivity. Furthermore, all serum samples had been heated to 56°C for 30 minutes prior to isolation of IgG. This process is known to inactivate most nucleases.

Correlation of anti-RT activity with clinical outcome was approached by a retrospective survey of the 16 asymptomatic HIV-seropositive individuals presented in Fig. 1. All have been followed for a mean of 3 years, with a range of 2 to 5 years. Sequential serum specimens were obtained from each individual and stored at −70°C. At initiation of sample collection these persons corresponded to WR stage 0 (12 of 16) or WR stage 3 (4 of 16, no lymphadenopathy) in the Walter Reed Staging Classification (*15*). Of the 11 with anti-RT activity (≥50% inhibition at 10 μg of IgG), 6 have maintained these titers and 5 have lost this capacity. All of six with persistent inhibitors remain asymptomatic. All of four who lost this activity developed AIDS or ARC within 6 to 12 months after recognition of decreasing titer. Of the remaining five individuals lack-

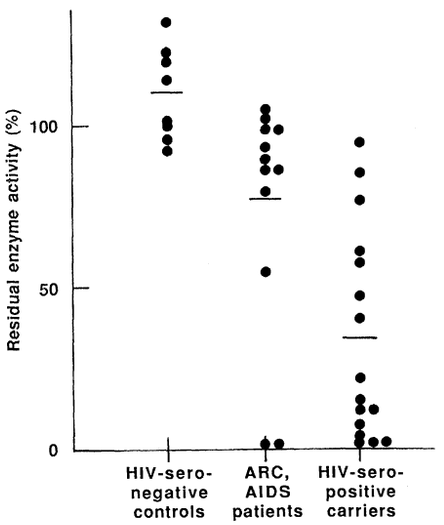

Fig. 1. Inhibition of HIV-associated RT activity by IgG. HIV virions, prepared from culture supernatants of $HIV_{HTLV\text{-}III_B}$-infected H9 lymphoblasts, were treated with virus-solubilizing buffer consisting of 0.8*M* NaCl, 0.5% Triton X-100, 1 m*M* dithiothreitol, 0.5 m*M* PMSF, 20% glycerol, and 50 m*M* tris (*p*H 7.9). Aliquots (25 μl) were incubated with 10 μg of total IgG, prepared from sera heated to 56°C for 30 minutes, and held at 4°C for 4 hours. Enzyme activity was then assayed. Template buffer (75 μl) consisting of 64 m*M* tris (*p*H 7.9), 11 m*M* $MgCl_2$, 1.1 m*M* dithiothreitol, 0.14 m*M* deoxyadenosine triphosphate, 7.5 mg/ml of poly(rA) · $oligo(dT)_{12\text{–}18}$, and 15 μ*M* [^{3}H]thymidine triphosphate were added to each virus sample. The reaction was run for 1 hour at 37°C with constant shaking and stopped with a solution of cold 10% trichloroacetic acid (TCA), 0.1*M* sodium pyrophosphate, and 2 mg/ml of yeast transfer RNA in 10 m*M* tris and 1 m*M* EDTA. Precipitates were collected on 26-mm fiber glass filters, washed with cold 5% TCA, dried, and counted. Enzyme activity of 100% represents [^{3}H]thymidine monophosphate incorporation in a reaction mixture containing phosphate-buffered saline (PBS) in place of IgG. IgGs obtained from HIV-seronegative asymptomatic controls, patients with AIDS, patients with ARC, and HIV-seropositive asymptomatic viral carriers were studied. Horizontal bars indicate mean residual RT activity for each group.

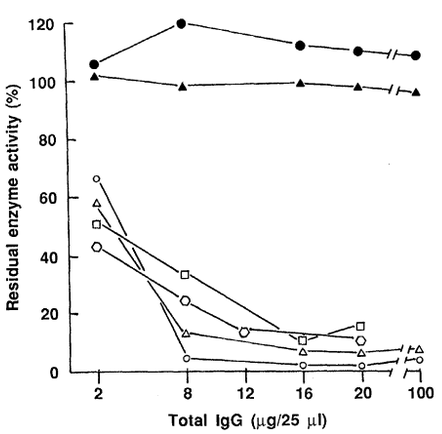

Fig. 2. Effect of increasing concentrations of total IgG prepared from heat-inactivated sera on HIV-associated RT activity. Assay conditions were as described in the legend to Fig. 1. Reaction mixture volumes were normalized with PBS. The closed symbols represent control IgGs, derived from an HIV-seronegative male (▲) and a female with HTLV-I–associated $CD4^+$ cell lymphoma (●). The open symbols represent IgG from HIV-seropositive asymptomatic carriers (○, △ and □) or a rabbit IgG to disrupted $HIV_{HTLV\text{-}III_B}$ virions (◯).

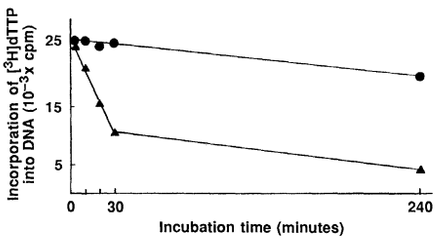

Fig. 3. Time course for inhibition of HIV RT activity. Solubilized virus was incubated with 10 μg of IgG from an HIV-seropositive asymptomatic carrier (▲) or with PBS (●) at 4°C for 10, 20, 30, or 240 minutes. Enzyme assays were performed, in duplicate, immediately after each incubation period, as described in Fig. 1.

Table 1. Specificity of the RT inhibitor of HIV. All enzyme assays were run in the presence of poly(rA) · oligo(dT)$_{12-18}$ as template primer, except as indicated. Conditions are listed in Fig. 1. All polymerases except that derived from HIV included 10 m*M* $MnCl_2$ in the reaction mixture in addition to $MgCl_2$. The results are expressed as the percentage inhibition of catalytic activity for the mean of the two experiments. Standard deviations were within 10% of the mean.

Source of IgG	Concentration (μg) of IgG	Polymerase source*					
		HIV	AMV	MoLV	FeLV	*Escherichia coli*	
						Poly(rA) · oligo(dT)	Poly(dA) · oligo(dT)
HIV-seropositive asymptomatic carrier	100	90.7	0	0	12.4	11.5	0
Rabbit antibody to HIV	70	86.4	0	0	30.4	0	0

*Purified RTs were purchased from Bethesda Research Laboratories (Moloney murine leukemia virus, MoLV) or Boehringer Mannheim (avian myeloblastosis virus, AMV, and *E. coli* DNA polymerase type I). RT from FeLV was prepared from culture supernatants of FL-74 cells infected with FeLV.

ing RT inhibitors, two have developed ARC, two remain clinically stable, and one was lost to follow-up.

Binding studies demonstrate the nearly universal immunogenicity of the endonuclease and RNA-dependent DNA polymerase products of HIV. Antibodies recognizing these proteins by immunoblotting appear independent of clinical status (*6, 7*), albeit the intensity of antigen-antibody complexes with other HIV antigens may correlate loosely with clinical stage of infection (*16*). To further characterize the proteins recognized by our inhibitory IgGs, we analyzed immunoprecipitates of [^{35}S]cysteine- and [^{35}S]methionine-labeled proteins from HIV-infected H9 lymphoblasts by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), as previously detailed (*17*). A representative sample of nine IgGs from HIV-seropositive subjects, including serial specimens >2 years apart obtained from three individuals, were tested.

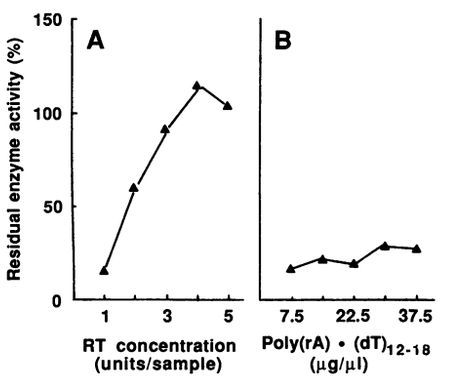

Fig. 4. Effect of increasing the concentration of disrupted HIV (**A**) or its template (**B**) on the inhibition by total IgG of reverse transcription of poly(rA) · oligo(dT). Solubilized HIV virions were incubated with 10 μg of IgG from an HIV-seropositive asymptomatic carrier at 4°C for 4 hours and then assayed as outlined in Fig. 1. The only variations were the final concentrations of disrupted virus (A) or template (B) in the reaction mixtures. One unit of RT activity corresponds to solubilization of approximately 1×10^3 tissue culture infectious doses of HIV.

The RT-associated protein p64 was recognized by all IgGs, the p51 RT product by eight of nine IgGs, and the p38 RT antigen by six of nine IgGs. As illustrated by eight selected samples in Fig. 5, these reactions occurred independently of the ability of the immunoglobulin to block HIV reverse transcriptase function. In agreement with another larger survey (*18*), there was also no difference in pattern of antibody recognition of HIV-related envelope (gp160 and gp120) or structural (p55, p24, and p17) antigens in symptomatic versus asymptomatic viral carriers (Fig. 5).

The origin of these inhibitory antibodies is unclear. They presumably arise from immunizations against enzyme from circulating disrupted virions or enzyme present on the infected cell membrane either as a mature protein or in the form of a group antigen-polymerase (*gag-pol*) polypeptide precursor. Catalytically inert *gag-pol* protein has been found on the surface of cells infected with mutants of murine leukemia virus blocked at late stages of viral assembly (*19*). RT activity in complexes without viral structure has also been observed in bone marrow cells transformed by reticuloendotheliosis virus (*20*). Polymerase-IgG binding without RT inactivation may have several explanations. IgGs recognizing divergent epitopes may be responsible for immunoprecipitation of *pol* gene products seen with samples from early asymptomatic (inhibitory to RT) as opposed to late symptomatic (noninhibitory) HIV-infected persons. For example, antibodies to peptides predicted from the 5′ end of mammalian retroviral *pol* genes may inhibit RT activity whereas antibodies to peptides from the midportion of the gene do not (*21*). Alternatively, decreased avidity of the RT-antibody interaction beyond the

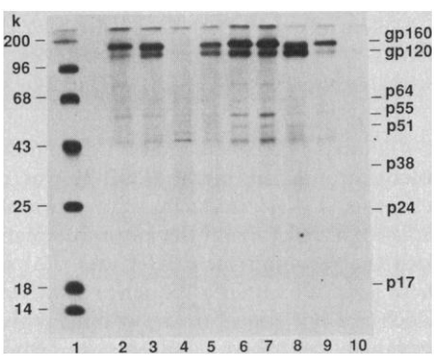

Fig. 5. Radioimmunoprecipitation of HIV proteins by selected IgGs. Lysates were prepared from $HIV_{HTLV\text{-}III_B}$-infected H9 lymphoblasts labeled with [^{35}S]methionine and [^{35}S]cysteine as previously detailed (*17*). They were precipitated with total IgG derived from sera of the individuals shown below.

Lane	Patient	Year serum was obtained	Diagnosis	IgG RT inhibitor
1	Molecular weight markers			
2	Bah	1983	Asymptomatic carrier	+
3	Bah	1985	AIDS	−
4	El	1983	Asymptomatic carrier	+
5	El	1985	AIDS	−
6	Dou	1983	AIDS	−
7	Gre	1983	Asymptomatic carrier	+
8	Bir	1985	ARC	−
9	Cha	1985	ARC	+
10	Control IgG from an HIV-seronegative individual			−

sensitivity of the radioimmunoprecipitation assay employed might be characteristic of the development of ARC or AIDS in certain individuals.

The possibility that antibodies to nonenvelope structures are effective in neutralizing HIV in vitro has been raised (*22*). Antibody to RT similarly might interfere with virion assembly or budding. Whether such a mechanism is responsible for the clinical correlations observed here is unknown. Most likely, IgGs with anti-RT capacity are surrogate markers for protective cellular immune responses or for other antibodies with reactivity against neutralizing envelope epitopes.

This latter area is controversial. Virus-neutralizing factors have been found in >50% of serum samples obtained from ARC and AIDS patients (*16*), and in a higher number of HIV-seropositive asymptomatic carriers (*23*). Clinical correlations in patients followed over time have been weak, however. The assays typically involve single HIV isolates as stock targets, although envelope variability is well documented among HIV isolates, even from a single individual (*24*). We attempted to link HIV neutralization in vitro with anti-RT activity, using IgG from ten of the asymptomatic carriers. We incubated 1×10^3 tissue culture infectious doses of stock $HIV_{HTLV\text{-}III_B}$ with 1 or 10 μg of IgG for 2 hours at 25°C. Phytohemagglutinin-activated human peripheral blood mononuclear cells (2×10^6) were then added in RPMI-1640 containing 10% fetal calf serum and 5% interleukin-2. Cultures were maintained for 18 hours at 37°C, the medium was changed, and HIV replication was assessed by RT determinations on days 7 and 14 after infection. Using ≥75% inhibition of enzyme activity as the criterion for neutralization, we found most samples effective at 10 μg, without regard to the patient's clinical status or the anti-RT capacity of the IgG.

In conclusion, circulating IgGs from certain individuals infected with HIV can block the catalytic activity of the viral polymerase in vitro. This effect is specific for the RT of HIV, although cross-reaction with HTLV-IV might be anticipated. RT-IgG binding, determined by radioimmunoprecipitation, did not correlate with RT suppression. Follow-up of a small cohort of asymptomatic HIV carriers revealed loss of this inhibitory capacity prior to development of clinical ARC or AIDS. If these data are reproduced in larger surveys of HIV-infected individuals, the assay could serve as a marker for disease progression, similar to the correlation of anti-RT activity with clinical status observed in other mammalian retroviral models.

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30 October 1986; accepted 3 February 1987

Human CSF-1: Molecular Cloning and Expression of 4-kb cDNA Encoding the Human Urinary Protein

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A 4-kilobase complementary DNA (cDNA) encoding human macrophage-specific colony-stimulating factor (CSF-1) was isolated. When introduced into mammalian cells, this cDNA directs the expression of CSF-1 that is structurally and functionally indistinguishable from the natural human urinary CSF-1. Direct structural analysis of both the recombinant CSF-1 and the purified human urinary protein revealed that these species contain a sequence of at least 40 amino acids at their carboxyl termini which are not found in the coding region of a 1.6-kilobase CSF-1 cDNA that was previously described. These results demonstrate that the human CSF-1 gene can be expressed to yield at least two different messenger RNA species that encode distinct but related forms of CSF-1.

THE PROLIFERATION OF HEMATOPOIETIC cells in culture requires the presence of one or more hematopoietic growth factors known as the colony-stimulating factors (CSFs) (*1*). Four subtypes of CSFs have been identified on the basis of hematopoietic cell lineages observed in colonies grown in the presence of the different factors. In the human system, the genes for all four of these subtypes have been cloned, thereby permitting analysis of the expression of these sequences in different cell types (*2–5*). In analyzing the expression of messenger RNAs (mRNAs) encoding

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