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Monoclonal anti-idiotypic antibody mimics the CD4 receptor and binds human immunodeficiency virus

(acquired immunodeficiency syndrome/receptor mimicry/T-lymphocyte surface molecule)

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ABSTRACT **A monoclonal anti-idiotypic (anti-Id) antibody, HF1.7, was generated against anti-Leu-3a, a mouse monoclonal antibody (mAb) specific for the CD4 molecule on human helper/inducer T lymphocytes. The anti-Id nature of HF1.7 was demonstrated by the following properties. *(i)* It reacted in a solid-phase immunoassay with anti-Leu-3a and not with a panel of irrelevant mouse mAbs. *(ii)* It partially inhibited the binding of anti-Leu-3a to $CD4^+$ T cells. *(iii)* It detected a common idiotype present on various anti-CD4 mAbs. Because the CD4 molecule represents the receptor site for human immunodeficiency virus (HIV), the etiologic viral agent of acquired immunodeficiency syndrome, we examined the ability of the anti-Id mAb HF1.7 to mimic CD4 and bind HIV. This anti-Id mAb reacted with HIV antigens in commercial HIV ELISAs and recognized HIV-infected human T cells but not uninfected cells when analyzed by flow cytofluorometry. Attesting further to the HIV specificity, the anti-Id mAb reacted with a recombinant gp160 peptide and a molecule of M_r 110,000–120,000 in immunoblot analysis of HIV-infected cell lysates. The anti-Id mAb also partially neutralized HIV infection of human T cells *in vitro*. These results strongly suggest that this anti-Id mAb mimics the CD4 antigenic determinants involved in binding to HIV.**

Acquired immunodeficiency syndrome (AIDS) is a devastating disease resulting from infection of many cellular components vital for the maintenance of immune homeostasis. Human immunodeficiency virus [HIV; also called human T-lymphotropic virus type III (HTLV-III), lymphadenopathy-associated virus (LAV), and AIDS-associated retrovirus (ARV)], the etiological agent of AIDS, is lymphotropic for cells expressing the CD4 molecule. HIV has been shown to infect not only the helper/inducer subset of T lymphocytes but also cells of the monocyte/macrophage lineage (1–4). *In vitro* infection by HIV can be effectively blocked by monoclonal antibodies (mAbs), such as anti-Leu-3a and OKT4A, directed against the CD4 target molecule (4–6). It has been shown recently (7) that HIV binds to the CD4 molecule via an envelope glycoprotein of M_r 110,000. These results imply that the CD4 antigenic determinants recognized by anti-Leu-3a and OKT4A either represent the site of attachment of HIV or are closely associated with it. Based on Jerne's idiotype network hypothesis (8), anti-idiotype (anti-Id, or Ab-2) against anti-Leu-3a or OKT4A (Ab-1) bearing the internal image should mimic the antigen (CD4) and bind to HIV envelope glycoprotein. This interaction in turn may inhibit the binding of HIV to CD4 on target cells and therefore could lead to viral inactivation.

A monoclonal anti-Id antibody, termed HF1.7, was generated against mAb anti-Leu-3a. HF1.7 exhibited the following properties. *(i)* It reacted in solid-phase enzyme-linked immunosorbent assay (ELISA) with anti-Leu-3a and not with a panel of irrelevant mouse mAbs. *(ii)* It partially inhibited the binding of anti-Leu-3a to $CD4^+$ T cells. *(iii)* It reacted with HIV antigens in commercial HTLV-III and LAV ELISAs. *(iv)* It reacted by viable membrane immunofluorescence assay with HIV-infected human T cells but not uninfected cells. *(v)* It bound to a molecule of M_r 110,000–120,000 in immunoblot analysis of HIV-infected-cell lysate. *(vi)* It bound a recombinant gp160 peptide by a double-antibody radioimmunoassay (RIA). *(vii)* The binding of anti-Leu-3a to its anti-Id mAb was inhibited by mAbs against CD4 but not by irrelevant mAbs. *(viii)* It partially neutralized HIV infection of human T cells *in vitro*. These results strongly suggest that mAb HF1.7 reacts with an idiotypic (Id) determinant on anti-Leu-3a and mimics part(s) of the CD4 molecule that represents the viral receptor for HIV and binds to HIV envelope glycoprotein. This binding may prevent the virus from attaching to target cells, resulting in viral neutralization. mAb HF1.7 may be an important reagent in the understanding of the molecular mechanism of HIV pathogenicity and in the development of diagnostic and therapeutic strategies.

MATERIALS AND METHODS

mAbs. The CD4-specific mAbs anti-Leu-3a (Becton Dickinson), OKT4A (Ortho Diagnostics), and anti-T4 (Coulter Immunology) were purchased from their manufacturer as purified immunoglobulins or were the gift of G. Thorton (Johnson and Johnson Biotechnology Center, La Jolla, CA). mAbs that recognize other lymphocyte phenotypic markers (Leu-1, Leu-2a, Leu-5b, Leu-8, Leu-M1) were purchased as purified immunoglobulins from Becton Dickinson.

Generation of Monoclonal Anti-Id Antibodies. Three- to five-week-old BALB/c mice were immunized intravenously with purified anti-Leu-3a mAb (30 μg per mouse) in 0.9% NaCl. Six injections were given at weekly intervals. Three days after the last injection, the mice were killed and their spleen cells were fused with the mouse myeloma cell line NS-1 as described previously (9). Supernatant fluids from wells with hybrid growth were screened for reactivity against HIV or anti-Leu-3a by an ELISA described below.

ELISAs. The HTLV-III ELISA (Electro-Nucleonics, Silver Spring, MD) and the LAV EIA (Genetic Systems, Seattle, WA) were done according to the manufacturers' specifications. Horseradish peroxidase-conjugated goat anti-mouse IgG antibodies (Vector Laboratories, Burlingame, CA) were substituted for goat anti-human IgG enzyme

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Abbreviations: AIDS, acquired immunodeficiency syndrome; FITC, fluorescein isothiocyanate; HIV, human immunodeficiency virus; Id, idiotype (idiotypic); mAb, monoclonal antibody; SV40 T antigen, simian virus 40 large tumor antigen; $TCID_{50}$, 50% tissue culture infective dose.

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Table 1. Reactivity of mAb HF1.7 with HIV antigens in ELISA

mAb	HTLV-III ELISA	LAV EIA	Psoralen- and UV-inactivated HIV
Negative control anti-Id	0.05 ± 0.01	0.06 ± 0.01	0.04 ± 0.01
Pooled AIDS serum*	1.20 ± 0.11	1.45 ± 0.15	1.01 ± 0.10
HF1.7 anti-Id	0.75 ± 0.08	1.20 ± 0.10	0.45 ± 0.03

Each value represents the mean ± SEM of triplicate determinations. See *Materials and Methods* for descriptions of the assays.
*Diluted 1:300.

conjugate. The ELISA using psoralen- and UV-inactivated HIV was done as described (10).

To determine the binding of HF1.7 to anti-Leu-3a, ascites fluid containing HF1.7 or a control anti-Id mAb (GB-2, which recognizes an idiotype associated with a mAb specific for hepatitis B surface antigen) was fractionated with 50%-saturated ammonium sulfate. The resulting immunoglobulin-containing precipitate was resuspended in borate-buffered saline (0.05 M, pH 8.2), and the concentration of antibody was determined, using an extinction coefficient of 14 for a 1% solution at 280 nm. Various concentrations of the anti-Id mAbs were adsorbed to triplicate wells of microtiter plates. After nonspecific sites were blocked by incubation with 10% normal goat serum in borate-buffered saline, either biotinylated anti-Leu-3a or a biotinylated control mAb specific for simian virus 40 large tumor antigen (SV40 T antigen) (11) was added. (The antibodies had been biotinylated at a concentration of 7 mg/ml, and a 1:1000 dilution in 10% normal goat serum was used in the assay.) After a 1-hr incubation at 37°C, unbound antibodies were removed by washing, and specific binding was detected by using avidin-horseradish peroxidase and followed by 2,2′-azinobis(3-ethylbenzthiazolinesulfonic acid) (ABTS) with H_2O_2. This assay was performed according to methods previously described (12).

Inhibition of Binding of Anti-Id mAb HF1.7 to mAb Anti-Leu-3a. Microtiter plates were coated with purified HF1.7 (500 ng per well). After blocking of nonspecific sites, 5 μg of various inhibitors were added to the anti-Id-coated wells for 1 hr. After incubation and washing to remove unbound antibodies, biotinylated anti-Leu-3a at a 1:1000 dilution was added and the ELISA was done as described above.

Immunofluorescence Staining. The immunofluorescence staining procedure was performed essentially as described (13). In brief, 10^6 cells were incubated with anti-Id HF1.7 or a negative antibody control of the same isotype for 30 min at 4°C, followed by fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG (Cappel Laboratories, Cochranville, PA) for an additional 30 min at 4°C. After incubation, the cells were washed, fixed in 0.37% formaldehyde, and analyzed by flow cytometry using a Becton Dickinson FACS analyzer interfaced to a BD Consort 30 (Becton Dickinson). To assess the inhibition of binding of anti-Leu-3a to $CD4^+$ cells by HF1.7, the human T-cell line CEM A3.01 was used (14). FITC-anti-Leu-3a (Becton Dickinson) was incubated with phosphate-buffered saline (PBS: 0.02 M, pH 7.4) or with PBS containing purified HF1.7 or control anti-Id mAb (10 μg) for 1 hr at 4°C and then was added to 5×10^5 A3.01 cells. The cells were incubated for 30 min at 4°C, washed twice, and analyzed on the FACS.

Immunoblot Analysis. The Bio-Rad Immunoblot System (Bio-Rad Laboratories) was used. In brief, nitrocellulose strips on which electrophoretically fractionated HIV antigens had been blotted were incubated in 20 mM Tris·HCl/150 mM NaCl, pH 7.4/1% bovine serum albumin/0.2% Tween 20 to block nonspecific sites. The strips then were treated with pooled human AIDS sera (1:100) or 3-fold concentrated hybridoma supernatants containing anti-Id antibodies overnight at 4°C. The strips were washed with Tris·HCl buffer to remove unbound antibodies. Human and mouse antibody reactivities were detected with alkaline phosphatase-conjugated goat anti-human immunoglobulin and anti-mouse immunoglobulin (Sigma), respectively. The substrate used was provided by Bio-Rad Laboratories.

Binding to Recombinant HIV Envelope Antigens. A recombinant gp160 peptide produced in the baculovirus expression-vector system and purified by lectin chromatography (Micro Gene Sys, West Haven, CT) was radiolabeled with ^{125}I by the chloramine-T reaction (15). Unreacted ^{125}I was removed by passage through a PD-10 column (Pharmacia). Approximately 92% of the radiolabel precipitated with protein in 10% trichloroacetic acid. A double-antibody RIA, similar to methods described in ref. 16, was performed using a hyperimmune rabbit anti-mouse IgG to precipitate all the mouse IgG that bound the ^{125}I-labeled gp160.

Neutralization of HIV Infection *in Vitro*. The neutralization assay was done as described (17). In brief, 1000 or 100 $TCID_{50}$

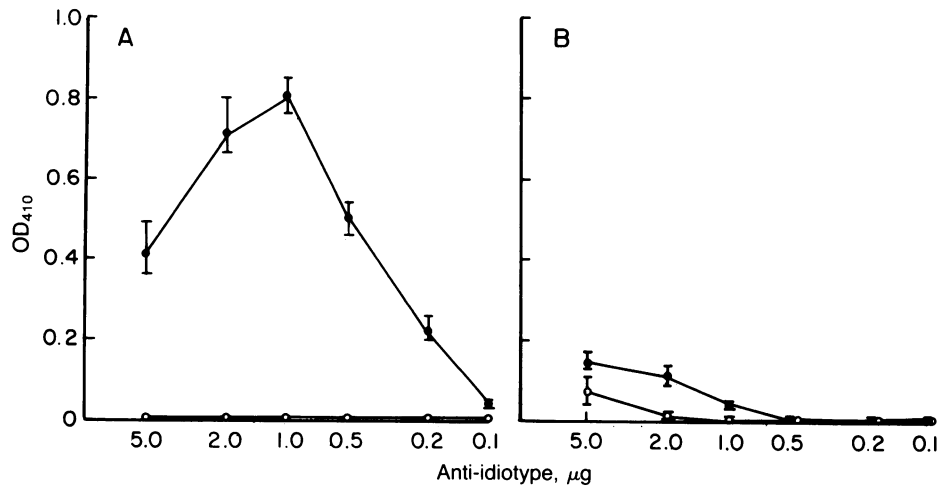

FIG. 1. Binding of biotinylated anti-Leu-3a to anti-Id mAb HF1.7. Microtiter wells were coated with various amounts of HF1.7 mAb (●) or GB-2 control anti-Id mAb of the same isotype (○) and treated with biotinylated anti-Leu-3a *(A)* or biotinylated antibodies to SV40 T antigen *(B)*.

(see below for definition) of HIV in 100 μl was incubated with 100 μl of HF1.7 or GB-2 control anti-Id or culture medium for 1 hr at 37°C. The concentrations of mAbs were adjusted to yield a final concentration of 0.5 mg/ml. After incubation, the treated HIV were added to 10^6 A3.01 cells and incubated at 37°C for 2 hr in the presence of Polybrene (Calbiochem) at 10 μg/ml. The cells were then washed and resuspended (10^6 per ml) in RPMI 1640 medium supplemented with 10% fetal bovine serum. At various times, aliquots of culture fluids were removed and reverse transcriptase (RNA-directed DNA polymerase, EC 2.7.7.49) activity was determined as described (17). Cell-free HIV was harvested from infected A3.01 cell culture and titrated on uninfected A3.01 cells, and the titer was expressed as 50% tissue culture infective dose ($TCID_{50}$).

RESULTS

Because our primary goal was to obtain mAbs reactive with HIV antigens, we chose to screen the hybrids by ELISA with HIV antigen-coated plates (Table 1). Among 389 hybrids tested, two were found that reacted in all three assays used. Thirty-five hybrids reacted with the immunizing antigen, mAb anti-Leu-3a (data not shown). One of the two hybridomas producing mAbs reactive with HIV antigens, designated HF1.7, was cloned twice by limiting dilution. The isotype of mAb HF1.7 was determined to be IgM.

To assess the specificity of HF1.7 binding, microtiter plates were coated with various concentrations of HF1.7 or a control mAb, GB-2, and allowed to react with biotinylated anti-Leu-3a (Fig. 1*A*) or biotinylated control mAb of the same isotype as anti-Leu-3a but recognizing SV40 T antigen (Fig. 1*B*). Anti-Id mAb HF1.7 specifically bound to the biotinylated anti-Leu-3a, whereas no binding was observed between the biotinylated anti-Leu-3a and the control anti-Id mAb. Neither HF1.7 nor the control anti-Id mAb bound to biotinylated control mAb specific for SV40 T antigen. Anti-Id HF1.7 did not react with a panel of irrelevant murine mAbs that included anti-Leu-1, -Leu-2a, -Leu-5b, -Leu-8, and -Leu-M1 or with normal mouse IgG.

At a concentration of 5 μg, the irrelevant mAbs failed to significantly inhibit the binding of anti-Leu-3a to its anti-Id mAb (range of inhibition 0–5%; Table 2). On the other hand, anti-Leu-3a and two other mAbs that recognize the CD4 molecule (OKT4A and anti-T4) were efficient inhibitors of the Id–anti-Id reaction. These data indicate that HF1.7 recognizes an Id determinant on anti-Leu-3a and that it may "mimic" CD4 in its binding to anti-CD4 mAbs. It is noteworthy that anti-Leu-3a, OKT4A, and anti-T4 all block *in vitro* infection by HIV (18). Thus, the ability to inhibit the Id–anti-Id reaction appears to correlate with the ability of the mAb to block HIV infection *in vitro*.

Table 2. Inhibition of binding of HF1.7 to anti-Leu-3a by various antibodies

Inhibitor	Isotype	Percent inhibition*
Anti-Leu-3a	IgG1,κ	94
OKT4A	IgG1,κ	91
Anti-T4	IgG1,κ	84
Anti-Leu-1	IgG2a,κ	0
Anti-Leu-2a	IgG1,κ	0
Anti-Leu-5b	IgG2a,κ	4
Anti-Leu-8	IgG2a,κ	5
Anti-Leu-M1	IgM,κ	3
Normal mouse IgG†		5

Each inhibitor was tested at a concentration of 5 μg per well.
*Mean of triplicate determinations.
†Purified from pooled normal BALB/c mouse serum.

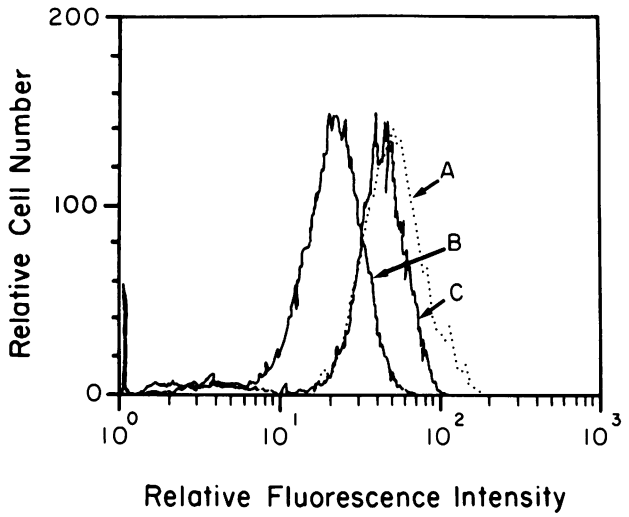

FIG. 2. Inhibition of binding of FITC-anti-Leu-3a to A3.01 cells by anti-Id mAb HF1.7. The A3.01 cells were stained with FITC-anti-Leu-3a in the presence of PBS (trace A) or PBS containing 10 μg of HF1.7 (trace B) or 10 μg of GB-2 (trace C).

The binding of mAb HF1.7 to anti-Leu-3a was further confirmed in another inhibition experiment using flow cytometry. Approximately 95% of cells of the human T-cell line A3.01 express surface CD4 as detected by immunofluorescence staining with anti-Leu-3a (14). Incubation of anti-Leu-3a with the HF1.7 anti-Id mAb resulted in a significant decrease in the fluorescence intensity of the anti-Leu-3a staining (Fig. 2). Anti-Leu-3a staining of the A3.01 cells was not significantly affected by prior incubation with the control anti-Id mAb. These data suggest that the anti-Id mAb can bind to anti-Leu-3a and partially inhibit anti-Leu-3a binding to surface CD4 present on human T cells. Therefore, the anti-Id mAb must recognize at least a portion of the antibody-combining site on anti-Leu-3a, based on its ability to inhibit binding to CD4 on human T cells. These characteristics further suggest that HF1.7 recognizes an Id determinant associated with the antibody-combining site on anti-Leu-3a.

To assess the expression of the antigen recognized by HF1.7 on the surface of HIV-infected cells by the anti-Id, an indirect immunofluorescence assay was performed on uninfected and continuously infected H9 cells (Fig. 3). Anti-Id staining of infected H9 cells resulted in a clear increase in fluorescence intensity, whereas uninfected H9 cells were not stained. Approximately 25% of HIV-infected

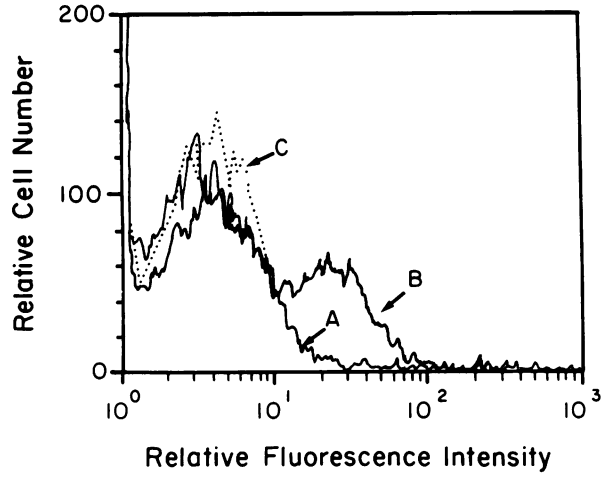

FIG. 3. Immunofluorescence profiles of uninfected (trace A) and HIV-infected (trace B) H9 cells stained with mAb HF1.7. Trace C shows GB-2 (negative control) staining of HIV-infected H9 cells.

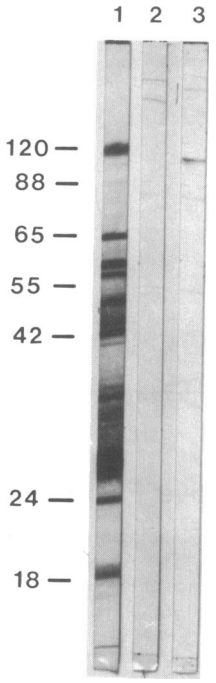

FIG. 4. Immunoblot analysis of HIV-infected cells. Blots were probed with pooled human AIDS serum diluted 1:100 (lane 1), mAb GB-2 (negative control; lane 2), or mAb HF1.7 (lane 3). Molecular weight markers ($M_r \times 10^{-3}$) are shown at left.

H9 cells were stained by the HF1.7 anti-Id. To determine the kinetics of the surface expression of the antigen recognized by the anti-Id on *in vitro* HIV-infected cells, we infected the human T-cell line A3.01 with HIV isolate NY-5 (19) and performed a viable-cell-membrane indirect immunofluorescence assay with anti-Id mAb on day 1 to day 7 of infection. The antigen recognized by HF1.7 was not detected until day 4 of infection, at which point 10–15% of the A3.01 cells were stained (data not shown). Thus, the anti-Id appears to recognize a determinant(s) present on HIV infected T cells.

To characterize the antigen reactive with HF1.7 anti-Id, we exposed nitrocellulose paper strips (Bio-Rad Immunoblot Assay), on which HIV antigens had been electroblotted, to HF1.7 mAb or to the negative control anti-Id. A pooled human AIDs serum was used as a positive control, at a dilution of 1:100. The human antisera recognized the characteristic HIV gag proteins p18 and p24 and the gag precursor p55 in addition to the envelope glycoproteins gp120 and gp41 (Fig. 4). HF1.7 anti-Id reacted with a band corresponding to gp120, with an approximate M_r between 110,000 and 120,000. No reactivity was found with the negative mAb control. The anti-Id recognized the HIV envelope glycoprotein gp120, which appears to represent the region where HIV binds the CD4 molecule.

To confirm the immunoblot analysis, a recombinant gp160 peptide produced in baculovirus was radiolabeled, and the percentage of this antigen that could be bound by the HF1.7 mAb was determined. At a 1:40 dilution of delipidated ascites fluid, 41% of the gp160 was bound with the anti-Id mAb. The control anti-Id preparation, GB-2, bound only 6% of the ^{125}I-labeled gp160 at a similar dilution of ascites. Excess unlabeled gp160 (10 μg) inhibited the binding of the HF1.7 mAb to ^{125}I-labeled gp160 by >95% (data not shown). These data indicate that the anti-Id mAb HF1.7 can bind the envelope glycoprotein of HIV.

The ability of HF1.7 mAb to inactivate HIV was assessed in an *in vitro* neutralization assay described previously (17). HIV replication was determined by measuring the reverse transcriptase activity in the culture supernatant fluids (Table 3). Reverse transcriptase activity was inhibited in cultures treated with HF1.7 anti-Id in a viral-dose-dependent fashion. The most pronounced inhibition of viral replication was observed on day 7 of culture, when 58% and 90% inhibition of reverse transcriptase activity was observed with 1000 and 100 $TCID_{50}$ of HIV, respectively. By day 9 of culture, the reduction of reverse transcriptase activity in HF1.7 treated cultures declined to 44% and 80% with 1000 and 100 $TCID_{50}$ of HIV, respectively. In contrast, GB-2-treated cultures produced approximately the same reverse transcriptase activity as that detected in medium-treated cultures. The increased reverse transcriptase activity in cultures treated with HF1.7 mAb on day 9 of culture presumably resulted from replication of HIV that escaped inactivation.

DISCUSSION

The causative agent of AIDS, HIV, primarily infects target cells that express the CD4 molecule. Antibodies, such as anti-Leu-3a and OKT4A, directed against the CD4 molecule effectively block the *in vitro* infectivity of HIV, presumably by competing with viral receptors. By utilizing anti-Leu-3a as the immunogen and selecting the resulting antibodies based on their ability to bind HIV antigens, we have generated an anti-Id mAb termed HF1.7, which appeared to "mimic" the CD4 determinant(s) involved in binding to HIV. HF1.7 was specific for anti-Leu-3a; it did not bind to any of a panel of mouse mAbs with different specificities or to normal mouse IgG. HF1.7 recognized an Id determinant closely associated with the binding site of anti-Leu-3a, since it effectively blocked the binding of anti-Leu-3a to cells of the human T-cell line A3.01, 95% of which express the CD4 molecules. In viable-cell-membrane immunofluorescence assays, mAb HF1.7 bound to ≈25% of HIV-infected H9 cells but not to uninfected cells; this observation suggests that the antigenic determinant detected by HF1.7 is a component of the HIV envelope and that it is exposed at the surface of infected lymphocytes.

Although no direct evidence is available to indicate that HF1.7 anti-Id bears an internal image, the observations that it *(i)* bound to anti-Leu-3a but not to irrelevant mouse mAbs, *(ii)* inhibited the binding of anti-Leu-3a to CD4, *(iii)* recognized an HIV envelope antigen with an approximate M_r of 110,000–120,000, and *(iv)* recognized a common Id shared by anti-CD4 mAbs that block HIV replication *in vitro* make it reasonable to speculate that the HF1.7 anti-Id bears an internal image that mimics the HIV viral receptor, the CD4 molecule. Radioimmunoprecipitation studies (7) have dem-

Table 3. Neutralization of HIV infection *in vitro* by mAb HF1.7

Virus concentration, $TCID_{50}$	Reverse transcriptase activity,* cpm					
	Day 7 of infection			Day 9 of infection		
	Medium	GB-2	HF1.7	Medium	GB-2	HF1.7
1000	31,562	30,110 (5)	12,760 (58)	160,156	161,058 (0)	91,026 (43)
100	4,094	3,569 (13)	369 (91)	54,516	53,476 (2)	10,836 (80)

*Each value represents the mean of duplicate cultures (see ref. 17 for reverse transcriptase assay). Numbers in parentheses indicate percent reduction in activity determined as [(cpm in medium alone − cpm in the presence of antibody)/cpm in medium alone] × 100.

onstrated binding of CD4 to a HIV envelope glycoprotein molecule of M_r 110,000.

Although the *in vitro* neutralization of HIV infectivity by HF1.7 was not complete, at least with the doses of HIV and anti-Id employed, these studies suggest that an internal-image anti-Id that mimics the viral receptor for HIV on susceptible T cells can partially inhibit viral replication. It is noteworthy that an anti-Id mAb recognizes only a single antigenic determinant on the viral envelope and may not be efficient at completely neutralizing viral infectivity. Similarly, the anti-Id mAb bound only 41% of a recombinant gp160 protein. These facts also suggest that the affinity of this anti-Id mAb for HIV antigens may be low. A pool of several anti-Id mAbs that recognize several sites on the viral envelope or a polyclonal anti-Id response may be more efficient in inhibiting viral replication and specific binding to the envelope glycoprotein. Recently, it was shown (20) that rabbit polyclonal anti-Id antibodies against anti-CD4 mAbs failed to bind HIV or inhibit the binding of the anti-CD4 mAbs to $CD4^+$ T cells. These polyclonal anti-Id antibodies appeared to recognize noncombining-site private Id expressed only on the anti-CD4 mAb utilized as an immunogen. This kind of anti-Id antibody has been referred to as an Ab-2_α, rather than the internal-image type of anti-Id antibody, referred to as Ab-2_β (21), that we describe here.

Numerous studies have demonstrated that anti-Id antibodies can mimic various substances and bind biological receptors (for a review, see ref. 22). More important and relevant to this report is that anti-Id antibodies have been used to isolate and identify the mammalian reovirus receptor (23, 24) and to identify receptors that may bind the envelope glycoprotein gp70 from murine leukemogenic retroviruses (25). The anti-Id antibody that recognized the reovirus receptor was capable of neutralizing viral infection of neurons (26). Based on the previous studies, it appears reasonable to utilize anti-Id antibody that can mimic a receptor, such as CD4, and bind a virus (HIV) at the site on the virus where it interacts with its receptor. This binding to HIV by the anti-Id antibody might be expected to neutralize infectivity by blocking the viral sites of attachment to the receptor.

In addition, studies reviewed in refs. 27 and 28 have indicated the possible role of anti-Id as vaccines against infectious agents. Recently, the vaccine potential for anti-Id was demonstrated for hepatitis B virus in chimpanzees, the relevant animal model for human infection (29). Because the anti-Id described in the present report partially neutralized HIV infection *in vitro*, one might speculate that the induction of a polyclonal anti-Id response elicited by anti-Leu-3a immunization could represent a possible means for vaccination against HIV. The studies described herein demonstrate that an anti-Id can be produced that mimics the viral receptor for HIV and binds the virus. This binding of the anti-Id to HIV can inhibit viral replication *in vitro*. Such reagents may be useful in understanding the molecular mechanisms of HIV pathogenicity. Anti-Id may also be used to develop new strategies for diagnosis of HIV infection.

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