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Immunology

Internal-image anti-idiotype HIV-1gp120 antibody in human immunodeficiency virus 1 (HIV-1)-seropositive individuals with thrombocytopenia*

(AIDS/anti-CD4 antibody/immune complexes/platelets)

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ABSTRACT **Anti-CD4 antibody was found in 30% of human immunodeficiency virus (HIV-1)-seropositive thrombocytopenic patients compared with 5% of nonthrombocytopenic seropositive patients ($\chi^2 = 21.7$, $P < 0.001$) and was shown by the following observations to contain internal-image anti-idiotype antibody (Ab2) directed against the antibody (Ab1) to gp120, the HIV-1 envelope glycoprotein that binds to CD4: (*i*) affinity-purified anti-CD4 (Ab2) bound to affinity-purified anti-HIV-1gp120 (Ab1) on solid-phase radioimmunoassay, and binding could be blocked by recombinant CD4 (rCD4) as well as recombinant gp120 (rgp120); (*ii*) F(ab')$_2$ fragments of Ab1 inhibited the binding of Ab2 to rCD4; (*iii*) Ab2 inhibited the binding of Ab1 to HIV-1 beads; (*iv*) Ab2 inhibited the binding of Ab1 to gp120 on immunoblot; (*v*) Ab2 bound to the CD4 receptor on a CD4-bearing T-cell line, H9; (*vi*) Ab3 (anti-rgp120) could be produced *in vivo* by immunizing mice with Ab2, and binding of Ab3 to rgp120 could be blocked with rCD4; and (*vii*) three different Ab2 preparations bound to two different homologous Ab1 preparations. Ab1 or Ab2 alone did not bind to platelets, whereas the idiotype–anti-idiotype complex did bind to platelets in a concentration-dependent manner. Binding of the internal-image complex was 10-fold greater than that of a non-internal-image Ab1–Ab2 complex composed of anti-HIV-1gp120 and anti-anti-HIV-1gp120. Thus, patients with HIV-1 thrombocytopenia contain internal-image idiotype–anti-idiotype complexes that could be affecting CD4 cell number or function, inhibiting HIV-1 binding to CD4 cells or contributing to HIV-1 thrombocytopenia.**

Our laboratory has reported anti-F(ab')$_2$ antibodies against IgG in the sera and polyethylene glycol-precipitable serum immune complexes of thrombocytopenic human immunodeficiency virus 1 (HIV-1)-seropositive homosexuals and narcotic addicts that correlated with serum immune complex level (1). Since some antibodies had broad reactivity against F(ab')$_2$ fragments of autologous and homologous patients as well as controls whereas others had limited reactivity against one or two F(ab')$_2$ fragments, we postulated a mixture of public anti-F(ab')$_2$ antibodies as well as anti-idiotype antibodies in the sera of HIV-1-seropositive patients. We recently noted the presence of anti-idiotype antibody (Ab2) directed against the antibody to HIV-1 envelope glycoprotein gp120 (Ab1) and idiotype (Ab1)–anti-idiotype (Ab2) complexes in the same group of HIV-1-seropositive patients (2–4). Ab2 reactivity correlated with immune complex level as well as thrombocytopenia, suggesting that idiotype–anti-idiotype (Ab1–Ab2) complexes could be responsible for the thrombocytopenia. The idiotype–anti-idiotype reactivity appeared to be Ab2α, since binding of Ab1 to gp120 could not be blocked with Ab2. The purpose of this study was to determine whether internal-image idiotype–anti-idiotype complexes were also present and whether this could be contributing to the thrombocytopenia.

We reasoned that patients with antibody directed against the CD4 receptor for gp120 might have internal-image Ab2β reactivity for anti-HIV-1gp120 antibody (Ab1). We found anti-CD4 antibody in 30% of HIV-1-seropositive thrombocytopenic patients. Affinity-purified anti-CD4 antibody contains internal-image anti-idiotype (Ab2) reactivity for affinity-purified anti-gp120 (Ab1). The complex assembled by incubation of affinity-purified anti-CD4 (Ab2) with affinity-purified Ab1 binds to platelets, whereas the individual components do not (3).

MATERIALS AND METHODS

Preparation of Fragments and Antibodies. Purified IgG was prepared by DEAE ion-exchange chromatography (1, 2) from 20 HIV-1-seropositive thrombocytopenic patients [platelet count, 55,000 ± 26,000 per μl (mean ± SD)], 20 HIV-1 nonthrombocytopenic patients, and five healthy controls.

Affinity-purified anti-HIV-1gp120 (Ab1) was prepared by adsorption of purified IgG to Affi-Gel 10 (Bio-Rad) coupled to recombinant gp120 PB-I (rgp120) of the HIV-1 envelope glycoprotein (courtesy of S. D. Putney, Repligen, Cambridge, MA) (2). Eluted Ab1 reacted exclusively with the gp120 band on immunoblot.

Affinity-purified anti-anti-HIV-1gp120 (Ab2) was prepared by adsorption to Affi-Gel 10 coupled to anti-HIV-1gp120 (2).

Affinity-purified anti-CD4 (Ab2) was prepared by adsorption to Affi-Gel 10 coupled to recombinant CD4 (rCD4; courtesy of Biogen).

F(ab')$_2$ fragments were prepared by pepsin digestion and protein A affinity chromatography (1). The purity of F(ab')$_2$ was established by SDS/PAGE and an immunoassay with anti-human IgG Fc antibody.

Anti-CD4 Radioimmunoassay. One hundred nanograms of rCD4 (courtesy of S. Chamow, Genentech) in 0.1 M $NaHCO_3$ buffer (pH 9.5) was applied to plastic microtiter wells overnight at 4°C, washed with 3% nonfat milk in phosphate-buffered saline (PBS), and then incubated with IgG at four doubling dilutions in 3% nonfat milk starting at 2000 ng/ml for 1 hr at room temperature. Wells were washed with 3% nonfat milk, incubated with ^{125}I-labeled staphyloccocal protein A (120,000 cpm per well, 30 mCi/mg; 1 Ci = 37 GBq; Amersham–Searle) for 1 hr at room temperature. Wells were rewashed and assayed for radioactivity. A result was con-

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Abbreviations: gp120, 120-kDa HIV-1 glycoprotein envelope; rgp120, recombinant gp120; Ab1, anti-gp120; Ab2, anti-anti-gp120 (anti-idiotype antibody); Ab3, anti-Ab2 and anti-rgp120; rCD4, recombinant CD4; HIV-1, human immunodeficiency virus 1.

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sidered positive when the cpm obtained from the first dilution of the linear IgG curve was greater by 3 SDs than the mean of nine separate control IgG samples, simultaneously assayed—i.e., 1069 ± 109 (1 SD). Positive samples were 2–4 times the control mean.

Anti-gp120 Radioimmunoassay. Five hundred nanograms of rgp120 (courtesy of K. Steimer, Chiron) was applied to plastic microtiter wells overnight at 4°C in PBS, washed in 3% nonfat milk, and incubated with mouse sera at serial dilution for 1 hr at room temperature. Wells were washed, incubated with ^{125}I-labeled protein A (^{125}I-protein A), rewashed, and assayed for radioactivity.

Anti-gp120 and p24/25 ELISAs. Two hundred nanograms of gp120 or p24/25 (ERC BioServices, Rockville, MD) were applied to microtiter plates in 0.1 M bicarbonate buffer (pH 9.5) overnight at 4°C, blocked with 1% bovine serum albumin in PBS, and treated with a 1:20 dilution of control or patient sera for 1 hr at 37°C. Wells were washed, and adherent material was treated with alkaline phosphatase-conjugated F(ab')$_2$ anti-human IgG (γ chain-specific; Sigma) for 1 hr at 37°C and developed with substrate for 25 min at room temperature. An absorbance reading was considered positive when greater by 3 SDs than the mean of 14 control subjects (>0.024 for gp120 and >0.032 for p24/25), simultaneously run. Positive samples were 3–40 times the control mean.

Detection of Anti-HIV-1 Antibody. Anti-HIV-1 antibody was detected by solid-phase adsorption ELISA assay with the Abbott Kit (Abbott) (2).

Immunoblotting. Nitrocellulose strips containing the HIV-1 antigens were purchased from Epitope (Beaverton, OR), incubated with purified IgG or affinity-purified Ab1 and/or Ab2 at various concentrations overnight at 4°C with gentle rocking, washed with 5% nonfat milk in PBS containing 0.5% Tween 20 (Sigma), and treated with goat anti-human IgG at 1 μg/ml coupled to horseradish peroxidase (Kirkegaard and Perry Laboratories, Gaithersburg, MD) for 1 hr at room temperature followed by substrate for 30 min at room temperature.

Fluorescence Flow Cytometry. CD4-bearing H9 cells (1 × 10^6), a gift of William Borkowsky (New York University Medical Center), were incubated with 10 μg of affinity-purified anti-CD4 (Ab2) ± 3 μg of rCD4 or 10 μg of control IgG in 100 μl for 30 min at 0°C in RPMI 1640 medium (GIBCO), sedimented, washed in RPMI 1640, and treated with 4 μg of fluorescein-conjugated mouse anti-human IgG (heavy and light chain specific, Biomedia, Foster City, CA) for 30 min at 0°C. Cells were then washed, fixed with 2% paraformaldehyde in PBS, and analyzed with a Becton Dickinson FACScan cytometer equipped with a 15-mW argon laser emitting at 488 nm. Ten thousand events were accumulated.

Immunization of Mice with Anti-CD4 (Ab2). BALB/c mice were immunized i.p. with 50 μg of F(ab')$_2$ fragments of affinity-purified anti-CD4 (Ab2) in complete Freund's adjuvant, and boosters were administered twice at 3-week intervals with 50 μg in incomplete Freund's adjuvant. Animals were bled 3 days later, and their sera were assayed for anti-gp120 reactivity.

Binding of Affinity-Purified Antibodies (Ab1 and Ab2) and Their Complex to Platelets. Washed platelets were prepared from EDTA-treated blood (5), and 10^7 were applied to U-shaped wells of polyvinyl microtiter plates (Dynatech) for 1 hr at room temperature. Plates were blocked, washed three times in 3% nonfat milk/0.02% azide and treated with IgG. Nonadherent IgG was washed away; the adherent IgG was treated with ^{125}I-protein A, washed, and monitored for radioactivity.

RESULTS

Detection of Anti-CD4, Anti-gp120, and Anti-p24/25 Antibody in Sera of HIV-1-Seropositive Subjects. Anti-CD4 antibody was detected in 6 of 20 (30%) HIV-1 thrombocytopenic patients tested compared with 1 of 20 (5%) HIV-1 nonthrombocytopenic patients ($\chi^2 = 21.7$, $P < 0.001$). Anti-gp120 and anti-p24/25 antibody detection was similar in the same two groups of thrombocytopenic and nonthrombocytopenic patients: 80% vs. 89% and 85% vs. 83%, respectively; ELISA absorbance levels were similar as well (data not shown).

Affinity-Purified Anti-CD4 Antibody (Ab2) Binds to Affinity-Purified Anti-HIV-1gp120 (Ab1). Fig. 1 shows affinity-purified anti-CD4 (Ab2 of patient AA) reactivity with F(ab')$_2$ fragments of anti-HIV-1gp120 antibody (Ab1) of two different HIV-1-seropositive individuals (C and D) and not with two control F(ab')$_2$ fragments. Reactivity could be blocked with rgp120, suggesting that Ab2 may be the internal-image anti-idiotype for Ab1. Reactivity could not be blocked with an irrelevant protein, ovalbumin, at the highest concentration of inhibitor used. Similar results were obtained with two additional affinity-purified anti-CD4 (Ab2) preparations.

Fig. 2 shows the ability of rCD4 to block the same anti-CD4 (Ab2) reactivity for Ab1 as blocked by rgp120. Reactivity could not be blocked with ovalbumin. Similar results were obtained with two additional anti-CD4 (Ab2) preparations.

Anti-HIV-1gp120 (Ab1) Antibody Blocks the Binding of Anti-CD4 Antibody (Ab2) to rCD4. If anti-CD4 (Ab2) is the internal-image anti-idiotype for Ab1 to the CD4 binding site of gp120, then Ab1 should block the binding of Ab2 to its epitope, rCD4. Fig. 3 shows the ability of F(ab')$_2$ fragments of Ab1 to block the reactivity of anti-CD4 (Ab2) from two different HIV-1-seropositive individuals (AA and BB) for rCD4. Note the lack of blocking reactivity with control F(ab')$_2$ fragments and lack of reactivity of control IgG for rCD4.

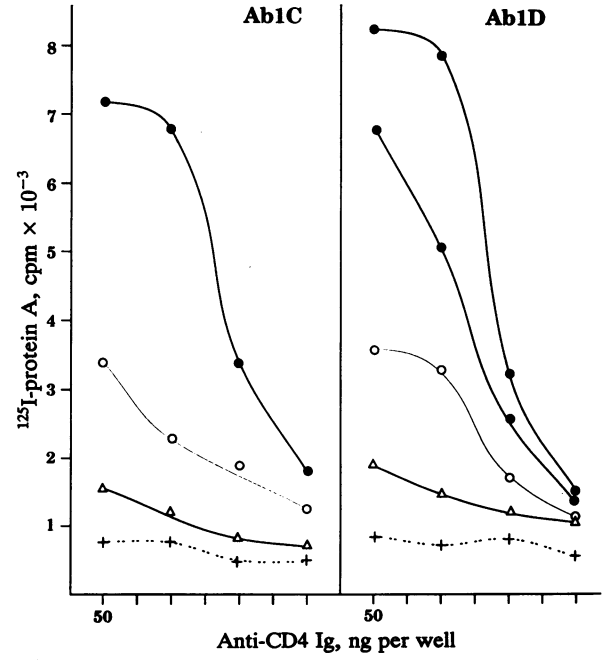

FIG. 1. Binding of anti-CD4 (Ab2) of patient AA to anti-HIV-1gp120 (Ab1) of patients C and D and inhibition of binding with rgp120. F(ab')$_2$ fragments of Ab1 were applied to microtiter wells at 2000 ng per well, washed, and blocked with 5% nonfat milk, and rgp120 was added at 0 (●), 100 (⊙), 200 (○), and 500 (△) ng per well. Ab2 was added at doubling dilutions starting at 50 ng per well, and binding was monitored with ^{125}I-protein A. +- -+, Ab2 with control F(ab')$_2$.

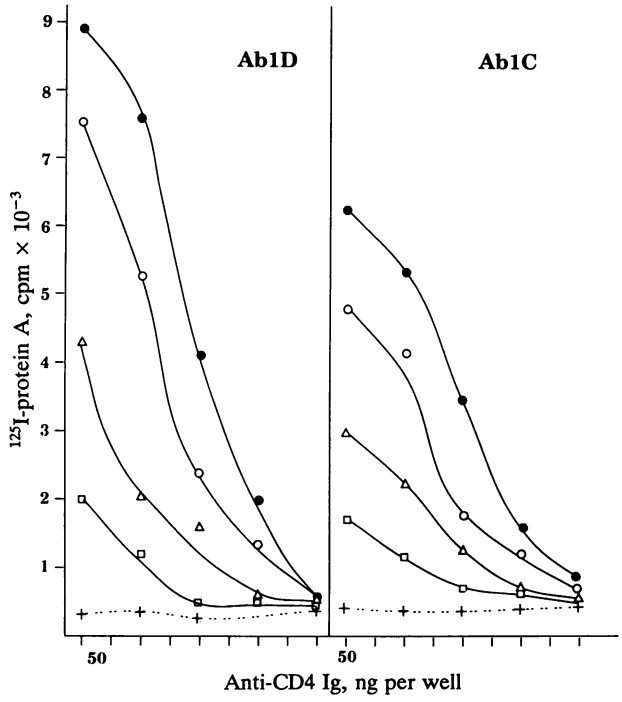

FIG. 2. Binding of anti-CD4 (Ab2) of patient AA to anti-HIV-1gp120 (Ab1) of patients C and D and inhibition of binding with rCD4. F(ab')$_2$ fragments of Ab1 were applied to microtiter wells at 2000 ng per well as above. Various concentrations of rCD4 were preincubated with 2 μg of Ab2 per ml at room temperature for 2 hr at CD4/Ab2 ratios of 0:1 (●), 1:2 (○), 1:1 (△), and 2:1 (□) prior to addition to Ab1. The preincubated mixture was added at doubling dilutions starting at an Ab2 concentration of 50 ng per well, and binding was monitored with ^{125}I-protein A. +- -+, Ab2 with control F(ab')$_2$.

Anti-CD4 Antibody (Ab2) Blocks the Binding of Anti-HIV-1gp120 (Ab1) to gp120. Internal-image Ab2 should block the binding of Ab1 to its epitope, gp120. Fig. 4 shows the ability of two different anti-CD4 (Ab2) preparations from HIV-1-seropositive individuals (AA and BB) to block the binding of Ab1 to HIV-1 beads. Note the lack of blocking with control IgG.

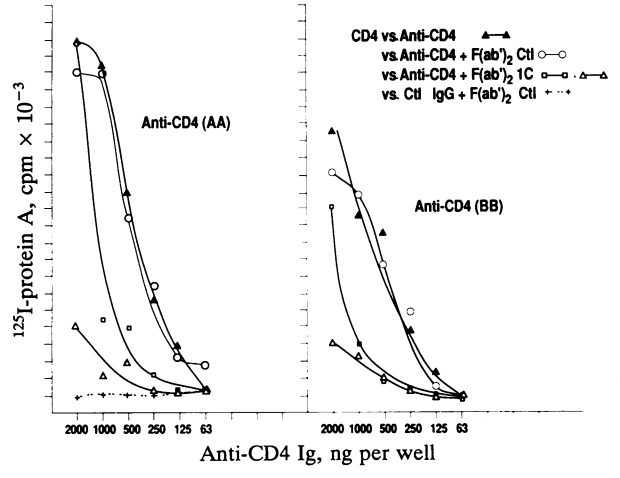

FIG. 3. Inhibition of binding of anti-CD4 (Ab2) to rCD4 with F(ab')$_2$ fragments of Ab1. rCD4 was applied to microtiter plates at 100 ng per well and treated with anti-CD4 (Ab2) (▲) or control Ig (+- -+) at serial dilutions starting at 2000 ng per well. Reactivity was monitored with ^{125}I-protein A. F(ab')$_2$ fragments of Ab1 (1C) were added at 200 (□) and 800 (△) ng per well, respectively; control (Ctl) F(ab')$_2$ was at 200 ng per well (○).

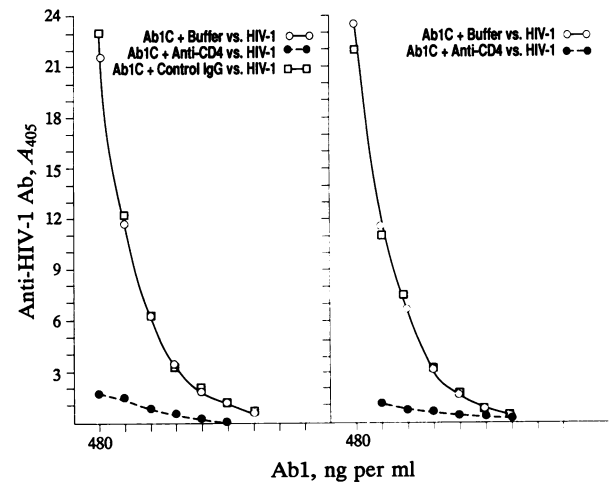

FIG. 4. Inhibition of binding of Ab1 to HIV-1 beads with anti-CD4 (Ab2). Anti-HIV-1gp120 (Ab1) was incubated with HIV-1 beads at serial dilutions starting at 480 ng/ml, and binding to beads was assayed by ELISA (○). Ab2 (●) or irrelevant IgG (□) was preincubated with Ab1 for 2 hr at room temperature at a ratio of 5:1 prior to the addition of the mixture to HIV-1 beads.

Similarly, Ab2 blocked the binding of Ab1 to gp120 on an immunoblot (Fig. 5), demonstrating the specificity of affinity-purified anti-HIV-1gp120 (Ab1) for gp120 (lanes 1 and 6–9) and the blocking of this binding by affinity-purified anti-CD4 (Ab2) (lanes 2–5). Note the lack of reactivity of anti-CD4 (Ab2) for gp120 (lane 10).

Binding of Anti-CD4 (Ab2) to CD4 on H9 Cells. Internal-image Ab2 should bind to CD4 on H9 cells if the conformation of CD4 on the cell surface is the same as rCD4. Fig. 6 shows

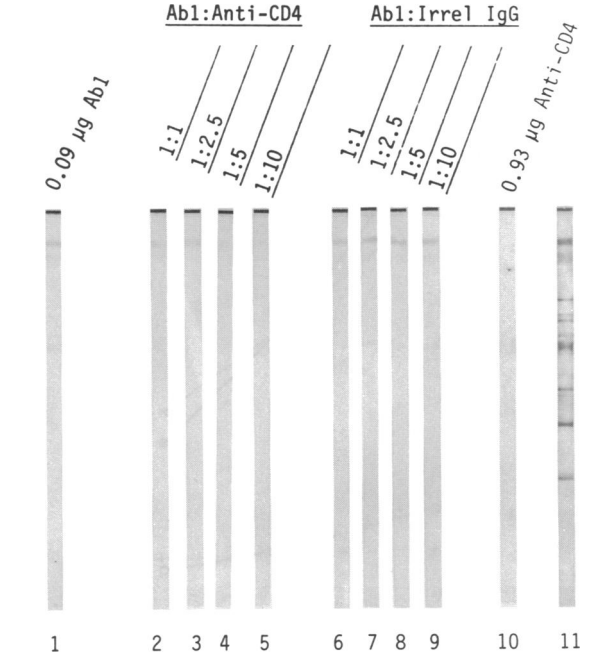

FIG. 5. Inhibition of binding of Ab1 to HIV-1gp120 by anti-CD4 (Ab2) on an immunoblot. Ab1, Ab2, and preincubated Ab1 with Ab2 were applied to nitrocellulose strips containing HIV-1 antigens. Reactivity was detected by ELISA. Lanes: 11, HIV-1-positive sera at a 1:50 dilution; 1, affinity-purified Ab1 at 0.09 μg/ml; 10, affinity-purified anti-CD4 (Ab2) at 0.9 μg/ml; 2, 3, 4, and 5, Ab1 at 0.09 μg/ml plus anti-CD4 (Ab2) at 0.09, 0.23, 0.45, and 0.9 μg/ml, respectively; 6, 7, 8, and 9, Ab1 at 0.09 μg/ml plus irrelevant (irrel) IgG at 0.09, 0.23, 0.45, and 0.9 μg/ml, respectively.

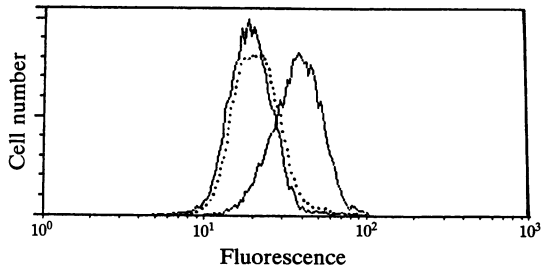

FIG. 6. Binding of anti-CD4 (Ab2) to CD4-bearing H9 cells. H9 cells (1×10^6) were incubated with 10 μg of control IgG (left curve, continuous line) or anti-CD4 (Ab2) (right curve) or 3 μg of rCD4 + 10 μg of anti-CD4 (left curve, dotted line), and binding was detected with fluorescein-labeled mouse anti-human IgG.

the binding of Ab2 to H9 cells compared with that of control IgG. The reaction was blocked with rCD4, indicating specificity of binding to the CD4 receptor. Similar binding results were obtained with a second anti-CD4 (Ab2).

Immunization of Mice with Affinity-Purified Anti-CD4 (Ab2). To prove that the anti-idiotype Ab2 is an internal image of gp120, it was necessary to produce anti-rgp120 (Ab3) *in vivo* by immunizing mice with Ab2. Fig. 7 shows the production of Ab3 in two such mice that react with rgp120 on radioimmunoassay. Note the lack of reactivity of control mouse serum for rgp120. Note the inhibition of anti-gp120 (Ab3) reactivity by prior incubation of rgp120 with rCD4.

Binding of Anti-HIV-1gp120 (Ab1) and Anti-CD4 (Ab2) to Platelets. We elected to determine whether the individual antibodies (Ab1 or Ab2) would independently cross-react with platelets, whether *in vitro* constructed complexes would bind to human platelets (platelets have an Fc receptor), and whether internal-image complexes would bind differently

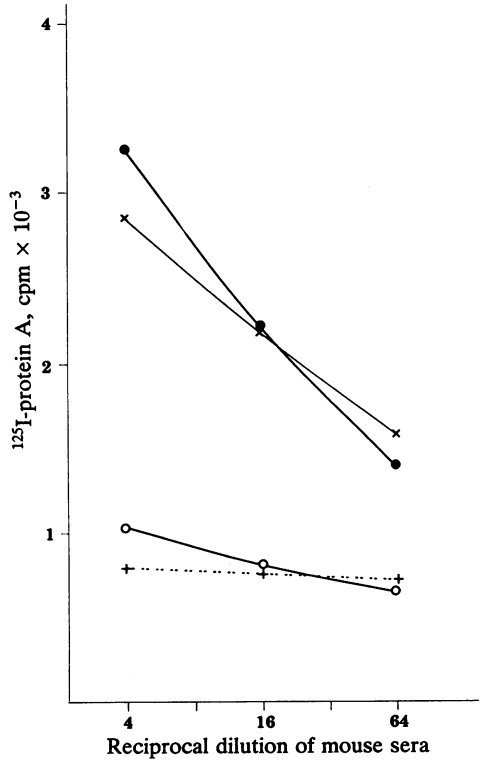

FIG. 7. Binding of Ab3 to rgp120. Two mice were immunized with affinity-purified Ab2, and their preimmune sera (+- -+) and immune sera (●, ×) were treated with 500 ng of rgp120 on microtiter plates. Ab3 could be blocked by addition of rCD4 at 500 ng per well (○).

from non-internal-image idiotype–anti-idiotype complexes. Fig. 8 (*right*) shows binding of Ab1–Ab2 internal-image complexes to platelets at various ratios of Ab1 to anti-CD4 (Ab2). Note the lack of reactivity of Ab1 alone or Ab2 alone, ruling out the possibility of cross-reactivity with platelets for these antibodies. Fig. 8 (*left*) shows similar data with Ab1–Ab2 non-internal-image IgG–anti-IgG complexes of anti-HIV-1gp120 and anti-anti-HIV-1gp120 [reactivity not blocked with rgp120 (2)]. Note the 10-fold greater reactivity of Ab1–Ab2 internal-image complexes for platelets compared with non-internal-image complexes. Similar results were obtained with a second affinity-purified anti-CD4 (Ab2).

DISCUSSION

Despite numerous experimental examples of anti-idiotype (Ab2) (6) production in animals immunized with xenogeneic, allogeneic, and syngeneic antigens [see reviews of 72 publications (7–9)], few examples are available for the presence and/or relevance of spontaneous anti-idiotype (Ab2) in a major clinical immunologic disorder.

Anti-anti-DNA autoantibodies have been reported in systemic lupus erythematosus sera during disease remission (10); anti-anti-acetylcholinesterase receptor autoantibodies have been reported in 40% of myasthenia gravis patients (11); anti-anti-casein autoantibodies and immune complexes have been reported in IgA-deficient subjects (12); and anti-anti-factor VIII autoantibody has been reported in a hemophiliac patient spontaneously recovering from a circulating factor VIII anticoagulant (13).

HIV-1-seropositive patients have polyclonal hypergammaglobulinemia and increased levels of circulating immune complexes (1, 2, 14–18). Since polyclonal activation (19) as well as immune complexes (20) can induce the formation of anti-idiotype (Ab2) autoantibodies, we postulated that anti-idiotype autoantibodies (Ab2) would be present in these patients. Indeed anti-HIV-1gp120 (Ab1) and anti-anti-HIV-1gp120 (Ab2) antibodies and complexes were found (2–4). However, since Ab2 did not block the binding of Ab1 to gp120, they were not internal-image Ab2β anti-idiotype but rather Ab2α (7).

Since anti-CD4 antibody has been reported in 5%–13% of HIV-1 seropositive patients (21–24), we postulated that pa-

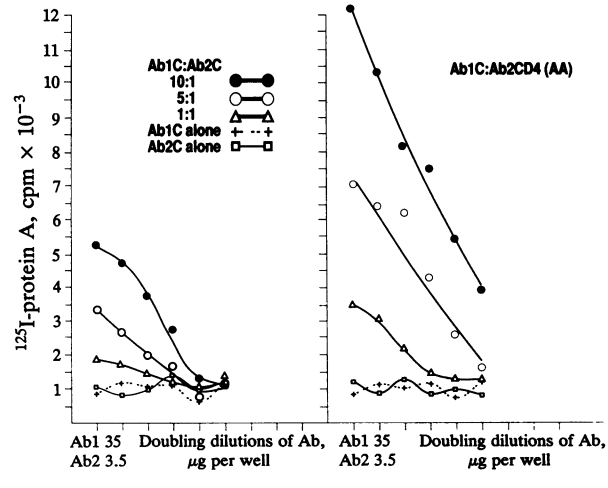

FIG. 8. Binding of Ab1, non-internal-image Ab2 (*Left*) and internal-image Ab2 (*Right*) to platelets. Platelets (10^7) were applied to microtiter plates, blocked, washed with 3% nonfat milk, and treated with Ab1 alone (+- -+), with Ab2 alone (□) or with a combination of both preincubated at various ratios [10:1 (●), 5:1 (○), and 1:1 (△)] at room temperature for 2 hr prior to addition to platelets. Abscissa Ab1/Ab2 concentrations are at ratios of 10:1. For other ratios, Ab1 was held constant and Ab2 was varied.

tients with antibody against the CD4 receptor for gp120 might have some antibody with internal-image Ab2β reactivity for HIV-1gp120 antibody (Ab1). Such proved to be the case. Thirty percent of thrombocytopenic HIV-1-seropositive patients had anti-CD4 antibodies. These were affinity-purified and shown to contain anti-anti-HIV-1gp120 (Ab2β): (*i*) anti-CD4 (Ab2) bound to anti-HIV-1gp120 (Ab1); (*ii*) Ab2 binding to Ab1 was blocked by rCD4 as well as by rgp120; (*iii*) $F(ab')_2$ fragments of Ab1 inhibited the binding of Ab2 to rCD4; (*iv*) Ab2 inhibited the binding of Ab1 to HIV-1 beads; (*v*) Ab2 inhibited the binding of Ab1 to gp120 on immunoblot; (*vi*) Ab2 bound to CD4 on the surface of a CD4-bearing T-cell line; (*vii*) Ab3 could be produced *in vivo* by immunizing mice with Ab2 and bound to rgp120; (*viii*) binding of Ab3 to rgp120 could be blocked with rCD4; and (*ix*) three different Ab2 preparations bound to two different homologous Ab1 preparations.

The presence of Ab2β antibodies capable of raising a heterologous anti-anti-idiotype (Ab3) against rgp120 raises the theoretical possibility that Ab2β could be acting as a protective immunogen or used as a vaccine (25, 26) in HIV-1 patients. Our observations support the work of others using xenogeneic systems wherein (*i*) a monoclonal anti-idiotype raised against anti-Leu3a mimicked the CD4 receptor by reacting with HIV-1gp120 on immunoblot and partially neutralized HIV-1 infection of human T cells *in vitro* (27); and (*ii*) a monoclonal Ab2β raised against a murine monoclonal antibody directed against the neutralization site of HIV-1gp120 (residues 308–322) was capable of raising an Ab3 in rabbits that reacted specifically with HIV-1gp120 and inhibited the infection of CEM cells by two different HIV-1 strains (HTLV-IIIB and HTLV-IIIMN) (28); and (*iii*) a monoclonal Ab2β raised against a rabbit anti-gp120 antibody (which inhibits syncytium formation and is specific for residues 503–535) was capable of raising an Ab3 in rabbits that reacted with gp120 via the epitope formed by residues 503–535 (29).

There are three possible clinically relevant aspects to these observations. (*i*) Anti-CD4 (Ab2β) antibody could modulate CD4 function or explain the low CD4 T-cell counts in some HIV-1 patients. Most CD4 T cells are not infected with virus (30). (*ii*) Anti-CD4 (Ab2β) antibody could block the entry of HIV-1 into T cells with the CD4 receptor as does Leu3a, a monoclonal antibody against the gp120-binding site on the CD4 receptor (31); preliminary evidence indicates inhibition of HIV-1 binding to a CD4-bearing T-cell line (HBP) by an HIV-1-ITP patient's affinity-purified anti-CD4 Ab. (*iii*) Anti-CD4 (Ab2β) antibody could contribute to the markedly elevated circulating immune complex level, noted particularly in HIV-1-thrombocytopenic patients (14, 16–18). Since these complexes are found on the platelets of these patients, they may be contributing to their increased peripheral destruction by macrophages. In this respect, we have recently induced platelet monocyte (U937 cells) rosette formation with polyethylene glycol-precipitable serum immune complexes of HIV-1-ITP patients that contain anti-idiotype anti-HIV-1gp120 complexes (4). This was 3.5-fold more than basal rosette formation in the presence of polyethylene glycol precipitates obtained from control sera (32). Therefore, it is of particular interest that binding of internal-image idiotype–anti-idiotype complexes to platelets is 10 times that of non-internal-image Ab1–Ab2 complexes and that the incidence of anti-CD4 Ab in thrombocytopenic HIV-1 patients is 6 times that in nonthrombocytopenic patients.

Our data indicate the presence of apparent endogenous internal-image Ab2β in 30% of HIV-1 thrombocytopenic patients. This is probably a low estimate, since bound anti-CD4 and immune complex-trapped anti-CD4 would not be detectable by the assay used. It is possible that this internal-image Ab2β may be contributing to disease manifestations of HIV-1-infected patients by modulating CD4 cell function or viability, affecting HIV-1 adherence to CD4 cells, acting as an immunogen for the production of neutralizing anti-gp120, or inducing thrombocytopenia via deposition of idiotype–anti-idiotype complexes on platelets.

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