Autoimmune Anti-HIV-1gp120 Antibody with Antiidiotype-like Activity in Sera and Immune Complexes of HIV-1-related Immunologic Thrombocytopenia

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Abstract

Autoimmune antiidiotype-like antibody (Ab2) directed against anti-HIV-1gp120 (Ab1) was found in high titer in the sera of 10 consecutive homosexual and 11 narcotic addict HIV-1-related immunologic thrombocytopenia (HIV-1-ITP) patients, was barely detectable in 10 nonthrombocytopenic HIV-1 sero-positive individuals, and was not detectable in 5 normal subjects by use of a solid-phase RIA. Reactivity of autologous Ab2 for Ab1 was 4–120-fold greater than Ab2 for homologous Ab1. Affinity-purified Ab2 did not block the binding of affinity-purified Ab1 to its HIV-1gp120 epitopes on immunoblot, indicating the absence of "internal image" antiidiotype. Both Ab1 and Ab2 are precipitable from sera with polyethylene glycol (PEG) and present in a macromolecular complex that is excluded by gel filtration on G200 and contains IgG, IgM, C3, and the anti-F(ab')2 antiidiotype-like complex. PEG-precipitable complexes bind to platelets in a saturation-dependent manner. Neither affinity-purified Ab1 nor Ab2 binds to platelets. However, the combination of Ab1 and Ab2 (preincubated for 2 h at 22°C) binds to platelets in a saturation-dependent manner at an optimum ratio range of 10–20:1. Ab2 reactivity correlates with serum PEG–precipitable immune complex level ($r = 0.91$; $P < 0.001$) and with thrombocytopenia ($r = 0.89$; $P < 0.001$). We suggest that the anti-HIV-1gp120 antiidiotype-like complex contributes to the markedly elevated platelet Ig and C3 level of HIV-1-ITP patients and propose that this may contribute to their thrombocytopenia. (*J. Clin. Invest.* 1992. 89:356–364.)
Key words: HIV-1-ITP • platelets • immune complexes • anti-F(ab')2 antibody • antiidiotype HIV-1gp120 antibody

Introduction

Immunologic thrombocytopenia (ITP)[1] is a frequent complication of HIV-1 infection in homosexuals (1), intravenous narcotic addicts (2), and hemophiliacs receiving antihemophilic factor concentrates (3). Platelet immune complex deposition (4–6) and specific antiplatelet antibody (2, 7–9) have been reported as possible etiologic agents.

We have previously reported the presence of anti-$F(ab')_2$ antibodies in the sera and polyethylene glycol (PEG)-precipitable immune complexes of HIV-1-ITP patients that correlated with the level of PEG-precipitable immune complexes (5). In addition, we found anti-HIV-1 antibody on platelets of HIV-1-ITP patients that eluted as a high-molecular-weight complex on gel filtration (6). Since HIV-1 antigen or proviral DNA was not detectable in platelet eluates or extracts, we postulated the presence of an autoantiidiotype to anti-HIV-1 antibody.

In this report we use a highly sensitive quantitative immunoassay to document the presence of autoimmune antiidiotype-like antibody (Ab2) against $F(ab')_2$ fragments of affinity-purified anti-HIV-1gp120 antibody (Ab1) in the sera of 10 homosexual and 11 narcotic addict HIV-1-ITP patients. Both Ab1 and Ab2 are PEG-precipitable and present within a macromolecular complex containing IgG, IgM, and C3. Ab2 reactivity correlates inversely with platelet count. Although neither Ab1 nor Ab2 alone binds to platelets, the complex of both antibodies binds in a saturation-dependent manner.

Methods

Population. The population studied consisted of 59 HIV-1-seropositive patients: 29 homosexuals (24 with idiopathic thrombocytopenia [HSITP] and 5 with normal platelet counts), 30 intravenous narcotic addicts (25 with idiopathic thrombocytopenia [NITP] and 5 with normal platelet counts), 14 classic autoimmune thrombocytopenia (ATP) patients, and 14 healthy control subjects.

Purified IgG. Purified IgG was prepared from serum by ion exchange chromatography (5).

$F(ab')_2$ fragments. $F(ab')_2$ fragments were prepared from purified IgG as previously described (5): pepsin digestion, followed by dialysis against PBS, followed by affinity chromatography on insoluble staphylococcal protein A. The identity of the nonadherent purified $F(ab')_2$ was verified by SDS-PAGE as well as by immunoassay performed with rabbit anti-human IgG-Fc antibody (5).

Immune complexes. Immune complexes were prepared from serum by PEG precipitation (5). Precipitates were dissolved in one-fifth the usual volume, ~ 2–4 mg/ml. Immune complexes were gel filtered (see below) and the exclusion volume isotopically labeled with ^{125}I by the Iodogen method (Sigma Chemical Co., St. Louis, MO).

Gel filtration. Gel filtration was performed on Sephadex G-200 as previously described (4). Effluents were assayed for protein at 280 nm as well as ELISA HIV-1 OD at 405 nm.

HIV-1 antibody. HIV-1 antibody was detected by a solid-phase absorption, ELISA assay using the HIV Ab HIV-1 EIA kit (Abbott Laboratories, North Chicago, IL), as recommended by the manufacturer. Briefly, 5–10 serial dilutions of sample were incubated with HIV-1-coated beads at 40°C for 1 h, washed in water, incubated with anti-human IgG (H + L) coupled to horseradish peroxidase for 2 h at 40°C, washed, and incubated with substrate for 30 min at room temperature; the reaction was stopped with 1 N H_2SO_4. The linear portion of the

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1. *Abbreviations used in this paper:* Ab1, anti-HIV-1gp120 antibody; Ab2, antiidiotype-like antibody; ATP, autoimmune thrombocytopenia; HIV-1-ITP, HIV-1-related ITP; HSITP, homosexual patient with ITP; ITP, immunologic thrombocytopenia; NITP; narcotic drug addict patient with ITP.

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logarithmic curve of HIV OD versus dilution was utilized for test measurements.

Affinity chromatography of PEG-precipitable immune complexes. 1 ml of immune complexes, solubilized in PBS–0.02% azide (0.7 mg/ml), was applied to 1 ml of each of the following insoluble slurry: staphylococcal protein A on Sepharose CL4B (Sigma Chemical Co.); rabbit anti-IgM (μ-chain specific, Organon Teknika, Westchester, PA), 4 mg coupled to 1 ml of Affi-Gel 10; and goat anti-human anti-C3 (Organon Teknika), 2.4 mg coupled to 1 ml of Affi-Gel 10 (Bio-Rad Laboratories, Richmond, CA). Slurries were equilibrated in PBS before addition of sample and gently rocked overnight at 4°C. The slurry was then centrifuged at 100 *g* for 10 min at room temperature and the supernatant removed for testing. Bound material was washed five times with PBS, five times with PBS–0.2 M NaCl, and another five times with PBS; eluted with 0.1 M glycine buffer, pH 2.5; and neutralized with 1 M Tris buffer. Effluent samples were compared with original samples with respect to HIV-1 OD reactivity and binding to platelets. The fraction of the antibody removed by adsorption was calculated by comparison of the slopes of the linear portion (five to six serial dilutions) of the binding curve before and after adsorption to the affinity matrix.

Binding of PEG-precipitable immune complexes or affinity-purified antibodies to platelets on microtiter plates. Washed platelets were prepared from EDTA-anticoagulated blood as described (10) and 10^7 platelets applied to U-shaped wells of a polyvinyl microtiter plate (Dynatech Laboratories, Inc., Alexandria, VA) for 1 h at room temperature. Plates were blocked and washed three times in 3% Blotto–0.02% azide (11) and then reacted with test sample. Nonadherent test sample was washed away; the adherent sample reacted with ^{125}I-labeled staphylococcal protein A and washed; and wells were monitored for reactivity (see below under RIA for cross-reactive Ab2).

Binding of ^{125}I-labeled PEG-precipitable immune complexes to platelets. Washed platelets (1.2×10^6) were applied to microtiter plates, blocked, and washed as above. They were then treated with serial dilutions of ^{125}I-labeled solubilized immune complex (8.4×10^6 cpm/μg) starting at 200 ng/well or a 100:1 mixture of nonradioactive/radioactive immune complex starting at 2,000 ng/well for 2 h at room temperature. Finally, wells were washed and monitored for radioactivity. Binding of ^{125}I-labeled solubilized immune complexes to blocked microtiter plates in the absence of platelets was negligible (50–100 cpm).

Binding of HIV-1 antibody of PEG-precipitable immune complexes to platelets in suspension. Washed platelets (3.75×10^8) were suspended in 250 μl of serially diluted solubilized PEG-precipitable immune complexes or 7 S IgG (starting at 0.5 mg/ml) and incubated for 3 h at 22°C, followed by overnight incubation at 4°C. Similar results are obtained in the absence of overnight incubation at 4°C. The suspension was then applied to 4 ml of a 20% sucrose cushion and centrifuged at 12,350 *g* for 10 min at 4°C to separate bound from unbound ligand. Control experiments, with addition of 0.5 mg/ml of immune complex to the sucrose cushion in the absence of platelets, revealed no detectable Ab1 activity at the tip of the test tube in 250 μl, after centrifugation. The sucrose was removed, the platelets were suspended in 250 μl of PBS, and the bound Ab1 eluted by addition of 125 μl of 150 mM H_3PO_4, 100 mM NaCl, and 1.5% BSA, pH 2.8 for 10 min, with shaking at 37°C. The acidified suspension was centrifuged at 20,000 *g* and neutralized with 2.5 M Tris buffer to pH 8.0. 50 μl of this solution was then assayed for Ab1 reactivity by HIV-1 ELISA assay.

Affinity-purified Ab1. Recombinant gp120 of the HIV-1 envelope glycoprotein was kindly supplied by Dr. Scott D. Putney (Repligen Corp., Cambridge, MA) and designated PB1-IIIB. 2 mg was coupled to Affi-Gel 10. The gel was incubated with ~ 20 mg of purified IgG from 1 ml of HIV-1–positive serum overnight at 4°C. The gel was then washed extensively with five bed volumes of 0.01 M PBS, pH 7.4, five washes of PBS–0.5 M NaCl, and five washes of PBS. Ab1 was eluted with 0.1 M glycine, pH 2.5, monitored by 280-nm absorption, and immediately neutralized to pH 7.4 with 1 M Tris buffer. 1 ml of serum with a titer of > 1:2,000 provided ~ 200–400 μg of antibody. Specificity for gp120 was verified by immunoblot on nitrocellulose strips impregnated with multiple HIV-1 antigens (Epitope, Beaverton, OR). Although multiple bands were noted with HIV-1–positive sera, only one band was noted at the gp120 region with Ab1 (6). A standard curve was constructed for HIV-1 OD versus IgG protein, which was linear with a log–log plot. The sp act of three different affinity-purified preparations averaged 0.050 OD/ng IgG (6).

Ab1 was also prepared from PEG-precipitable immune complexes by a strategy described for anti-F(ab')$_2$ antibodies (5). Briefly, the gp120 Affi-gel affinity column was acidified to pH 2.5 with 0.1 M glycine buffer and mixed with immune complexes similarly acidified. The slurry was slowly neutralized by dialysis overnight against PBS–0.02% azide. By this method the solid-phase gp120 competes for the dissociated Ab1 of the immune complex as the pH is raised. The solid-phase matrix was then washed and eluted as above.

Affinity-purified Ab2. Ab2 was prepared by coupling affinity-purified Ab1 (0.6 mg) to an Affi-Gel 10 column. Purified IgG from 1 ml of patients' sera was adsorbed, washed, and eluted as above. 1 ml provided ~ 20 μg of Ab2. Specificity was verified by reactivity with F(ab')$_2$ frag-

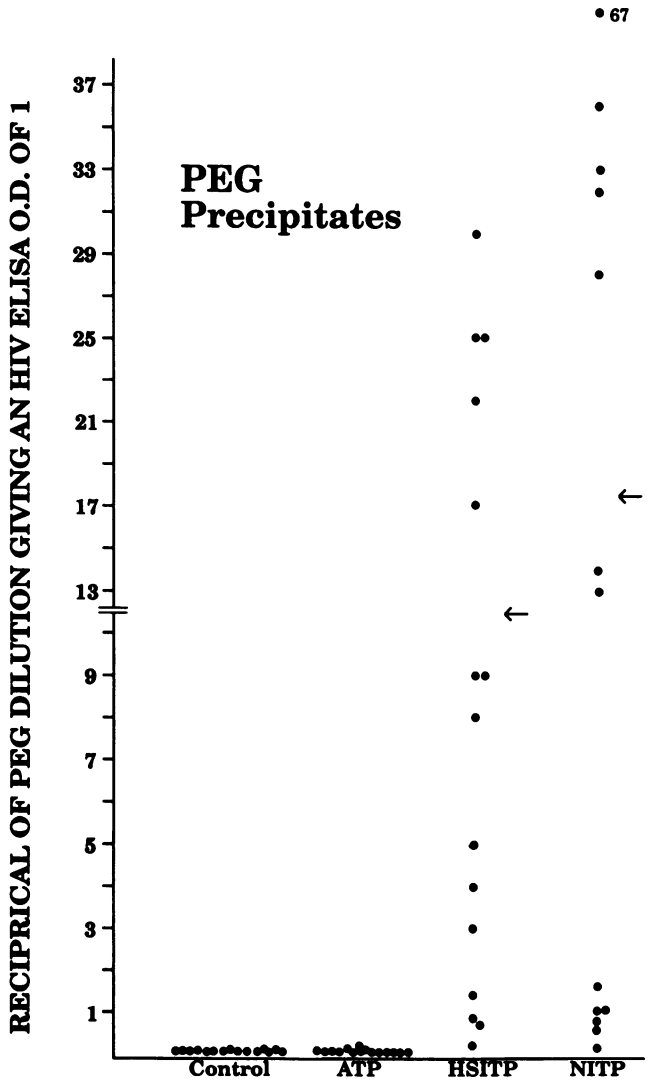

Figure 1. Ab1 of PEG-precipitable immune complexes from HIV-1-ITP patients (homosexuals [*HSITP*] and narcotic addicts [*NITP*]), classic autoimmune thrombocytopenic patients (*ATP*), and control subjects. PEG precipitates were solubilized and 2–4 mg/ml assayed for Ab1 by ELISA using HIV-1–coated beads. Readings > 2 OD were suitably diluted and reassayed (see Methods). The developing antibody was anti-human IgG coupled to horseradish peroxidase. After addition of substrate, OD was read at 405 nm. Arrows refer to mean value for the group.

ments of affinity-purified Ab1 but not with five control $F(ab')_2$ fragments using RIA (see below) and immunoblot against Ab1 bound to gp120 but not other HIV-1 antigens (6). Similar results were obtained if the IgG was first adsorbed to a pooled normal IgG affinity column and the supernatant employed for Ab2 reactivity.

Ab2 was also prepared from PEG-precipitable immune complexes by a strategy described for $F(ab')_2$ antibodies (5).

RIA for cross-reactive Ab2. The assay employed was similar to the anti-$F(ab')_2$ assay described previously (5). 50 μl of affinity-purified $F(ab')_2$ fragments of Ab1 (as well as all other reagents) was applied to microtiter wells at a concentration of 200 ng/well in PBS, blocked and washed in 3% Blotto, and then reacted with eight serial dilutions of either affinity-purified Ab2 or serum IgG starting at 200 ng/well. Unbound Ab2 was washed away with Blotto. The wells were then reacted with ^{125}I-labeled staphylococcal protein A (Amersham Corp., Arlington Heights, IL; 40 mCi/mg; ~ 60,000 cpm/well). Unbound ^{125}I-labeled protein A was washed away and the bottoms of the wells were removed with a hot wire cutter and assayed for radioactivity. Antibody reactivity was determined by extrapolating the linear portion of the Ab2 serial dilution curve (usually 5–6 data points) to the baseline *x*-axis. The baseline *x*-axis represents control reactivity, e.g., $F(ab')_2$ plus ^{125}I-labeled protein A. Thus the lower the concentration of Ab2 intersecting the *x*-axis, the greater the reactivity of Ab2.

Results

Ab1 in PEG-precipitable immune complexes of HIV-1-ITP patients. Fig. 1 demonstrates the presence of Ab1 antibody in 14 of 15 HSITP and 12 of 13 NITP. Note the markedly elevated values (> 2.0) in 11 of 15 HSITP and 7 of 13 ITP patients. Negative results were obtained in 15 control subjects as well as 15 chronic ATP patients.

Gel filtration of PEG-precipitable immune complexes of HIV-1-ITP patients. Fig. 2 depicts the gel filtration analysis of PEG-precipitable immune complexes of an NITP and HSITP patient. Note that > 90% of the 280-nm reactivity is in the void volume (exclusion fraction) of a G-200 Sephadex column. This high-molecular-weight region is associated with most of the Ab1 reactivity for the NITP and HSITP patient. Some 7S IgG Ab1 was also noted, which may represent partial dissociation of the complex because of dilution on the column or coprecipitation of some 7S IgG with PEG. Similar results were noted with two other NITP and two other HSITP patients (data not shown).

Binding of Ab1 of PEG-precipitable immune complexes to normal platelets. Fig. 3 demonstrates saturation-dependent binding of solubilized PEG-precipitable immune complexes to normal platelets, as measured by binding of ^{125}I-labeled immune complexes to platelets on microtiter plates (Fig. 3 *A*). Note inhibition of binding with a 100-fold excess of nonradioactive immune complex. Binding to platelets was also examined for Ab1 of the immune complex by elution of the bound Ab1 from platelets in suspension (Fig. 3 *B*). No binding was noted with gel-filtered 7S IgG Ab1 (Fig. 3 *B*).

Presence of Ab1 in a macromolecular complex of IgG, IgM, and C3. Table I depicts the macromolecular nature of the PEG-precipitable immune complex for four different patients. Thus, after incubation with insoluble protein A–Sepharose, > 98% of Ab1 reactivity and 100% of platelet-binding reactivity is removed; with insoluble anti–human IgM, > 94% Ab1 reactivity and 37–49% of platelet-binding reactivity is removed; with insoluble anti–human C3, > 99% Ab1 reactivity and 86–96% of platelet-binding reactivity is removed; with control Affi-Gel 10, 0–4% Ab1 reactivity and 0% platelet-binding reactivity is removed. Elution experiments of the bound complexes revealed 65–100% recovery of Ab1 and 98–103% recovery of platelet-binding reactivity for protein A and 82–96% recovery of Ab1 and 85–86% recovery of platelet-binding reactivity for anti-C3. With insoluble anti-IgM, 12–30% of platelet-binding activity could be eluted, resulting in a total recovery of 75–93% of total platelet-binding reactivity (i.e., eluted reactivity plus residual supernatant platelet-binding reactivity).

Ab2 against Ab1 in patient sera. Ab2 was demonstrated in the sera by the reactivity of affinity-purified Ab2 of three HIV-1-ITP patients with $F(ab')_2$ fragments of Ab1 from three HIV-1-ITP patients at the picogram level (Fig. 4 *A*). Ab2 did not react with $F(ab')_2$ fragments of three healthy control subjects or

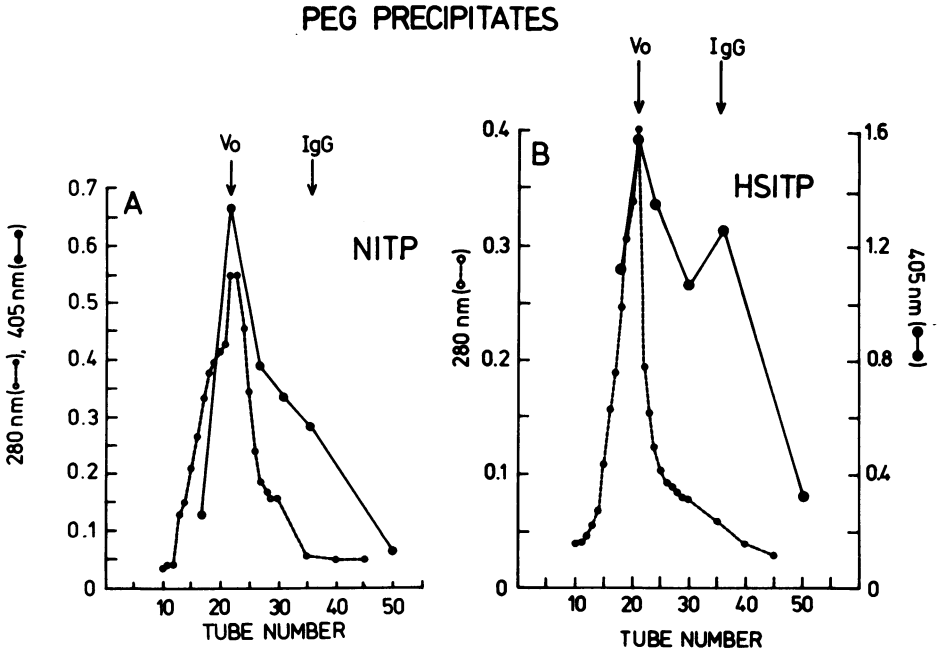

Figure 2. Effect of gel filtration on location of Ab1 activity in PEG-precipitable immune complexes. Solubilized immune complexes were applied to a sephadex G-200 column and the various effluents monitored for protein at 280 nm (o) and HIV-1 OD at 405 nm (●). Arrows refer to void volume (Vo) and 7S IgG locations. Note the presence of Ab1 activity in the high-molecular-weight Vo fraction of an NITP and HSITP patient.

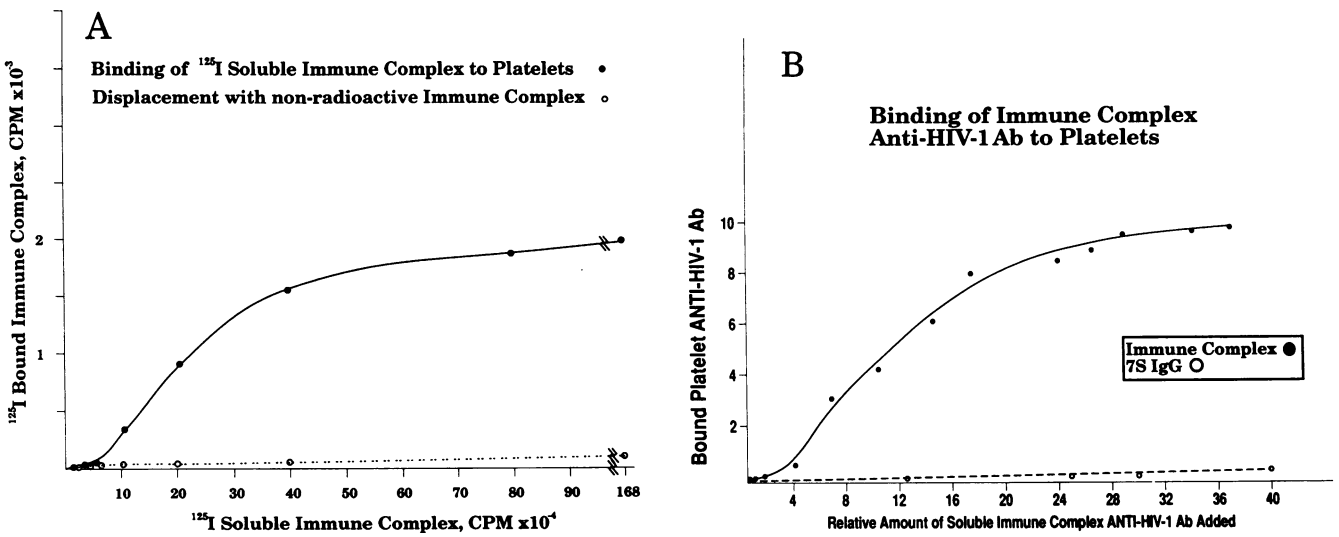

Figure 3. (*A*) Binding of ^{125}I-labeled, PEG-precipitable immune complex protein from patient NITP-EE to platelets. 1.2×10^6 washed platelets were applied to microtiter plates, blocked with Blotto, and then treated with serial dilutions of ^{125}I-solubilized immune complex (8.4×10^6 cpm/μg) starting at 200 ng/well (● —— ●) or a 100:1 mixture of nonradioactive/radioactive immune complex starting at 2,000 ng/well (○ —— ○) for 2 h at room temperature. Wells were then washed and monitored for radioactivity. (*B*) Binding of PEG-precipitable immune complex Ab1 from patient NITP-EE to platelets. 3.8×10^8 washed platelets were suspended in 250 μl of serially diluted, solubilized, PEG-precipitable immune complex (starting at 0.5 mg/ml; 36.9 reciprocal dilution, giving an HIV OD of 1/ml) or 7S Ig (starting at 0.5 mg/ml; > 40 reciprocal HIV dilution as above) and incubated for 3 h at 22° followed by overnight incubation at 4°. The suspension was then applied to a 20% sucrose cushion and centrifuged to separate bound from unbound ligand. Bound Ab1 was eluted with acid, neutralized, and assayed by ELISA beads as in Fig. 1 for Ab1.

from a patient with high titer anti-PLA^1 antibody. IgG from five control subjects and one patient with high-titer PLA^1 antibody did not react with the three F(ab')$_2$ fragments of Ab1 (data not shown). Of interest was the increased reactivity of autologous Ab2 with Ab1 in the three patients studied. This reactivity was 4–12-fold greater for patient A, 7-fold greater for patient C, and 10–120-fold greater for patient D compared with reactivity with homologous HIV-1-ITP patients' F(ab')$_2$ fragments.

Presence of Ab2 against Ab1 in PEG-precipitable immune complexes. Fig. 4 *B* demonstrates the reactivity of affinity-purified Ab2 from an NITP and an HSITP patient, respectively, with F(ab')$_2$ fragments of Ab1. These data are similar to those

Table I. Effect of Binding of PEG-precipitable Immune Complexes to Insoluble Staphylococcal Protein A, Anti-Igm, and Anti-C3 on Residual Anti-HIV-7 and Platelet-binding Reactivity

Patient	Residual supernatant reactivity (elutable reactivity)			
	Protein A	Anti-IgM	Anti-C3	Control Affi-Gel-10
		%		
HSITP-AA				
Anti-HIV-1	<1.0 (82)	4.9 (ND)	<1.0 (96)	96 (0)
Platelet binding	0 (103)	63 (12)	14.0 (86)	100 (0)
HSITP-BB				
Anti-HIV-1	ND	ND	<1.0 (82)	ND
Platelet binding	0 (100)	63 (30)	4.0 (ND)	100 (0)
NITP-CC				
Anti-HIV-1	1.1 (65)	5.4 (ND)	<1.0 (96)	107 (0)
Platelet binding	0 (102)	51 (28)	5.0 (85)	100 (0)
NITP-DD				
Anti-HIV-1	<1.0 (65)	2.0 (ND)	<1.0 (85)	103 (0)
Platelet binding	0 (98)	62 (14)	12 (86)	100 (0)

Solubilized, PEG-precipitable immune complexes were incubated with insoluble staphylococcal protein A, anti-human IgM and anti-human C3 antibody, as well as control Affi-Gel 10, overnight; and the residual supernatant was assayed for anti-HIV-1 as well as platelet-binding reactivity. Numbers in parentheses refer to percent acid-elutable reactivity from bound Affi-Gel. ND refers to not done.

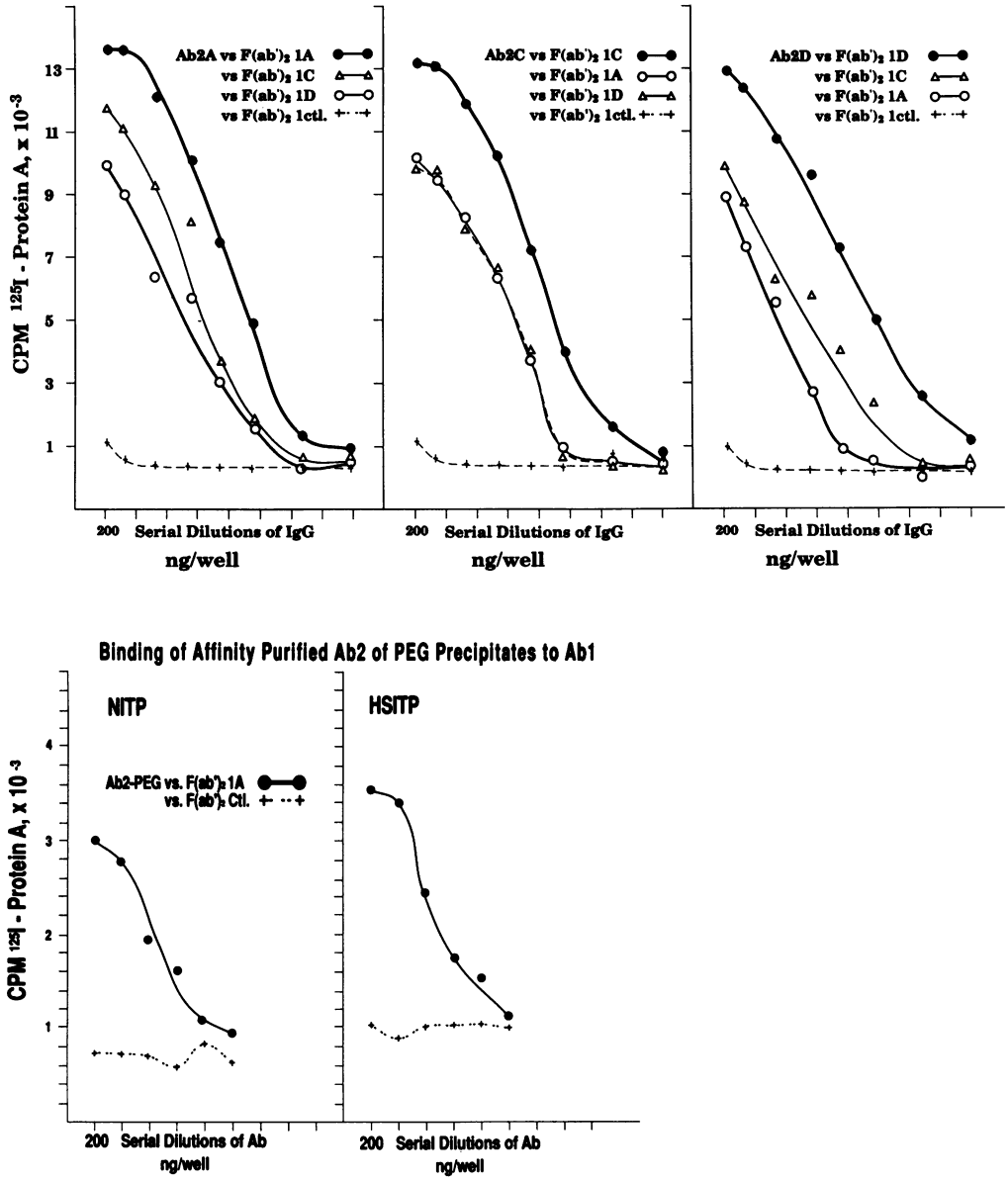

Figure 4. (*A*) Binding of affinity-purified Ab2 to $F(ab')_2$ fragments of affinity-purified Ab1. $F(ab')_2$ fragments of Ab1 from HSITP patients A and D and NITP patient C as well as control subject (1 ctl) were applied to microtiter plates at a concentration of 2,000 ng/well and blocked and washed with Blotto. Serial doubling dilutions of Ab2 starting at 200 ng/well were then applied. Unbound Ab2 was washed away with Blotto and bound Ab2 assayed with ^{125}I-staphylococcal protein A. (*B*) Binding of affinity-purified Ab2 of PEG precipitates to $F(ab')_2$ fragments of Ab1 from patient serum. Affinity-purified $F(ab')_2$ fragments of Ab1 from patient HSITP-A serum were applied to microtiter plates and then reacted with affinity-purified intact Ab2 from the PEG precipitate of patients NITP-R and HSITP-J.

obtained with Ab2 and Ab1 affinity purified from sera (Fig. 4 *A*).

Correlation of Ab2 with PEG-precipitable immune complexes and thrombocytopenia. Purified IgG from 10 NITP patients and 11 HSITP patients was tested for its reactivity with $F(ab')_2$ fragments of affinity-purified Ab1 from an NITP and an HSITP patient. All 21 IgG fractions reacted with both $F(ab')_2$ fragments, whereas 5 control IgG fractions showed no reactivity (Table II). HIV-1-ITP patients' Ab2 reactivity varied in HSITP patients from a detection sensitivity at 33–80 pg for patient J to 4,440 pg for patient I; in NITP patients the values ranged from 44–110 pg for patient U to 2,400–3,820 pg for patient Y. The degree of reactivity of the Ab2 of the individual patients was very similar with both Ab1 $F(ab')_2$ preparations tested. Similar studies were performed on nonthrombocytopenic (> 168,000/μl platelet count) HIV-1–seropositive homosexuals (five subjects) and narcotic addicts (five subjects). They were found to have barely detectable levels of Ab2 at concentrations of 20 to 200 ng.

A log–log correlation analysis of PEG-precipitable immune complexes versus Ab2 reactivity revealed a significant correlation for both homosexuals ($r = 0.95$, $P < 0.001$; 28 measurements on 14 individuals) and narcotic addicts ($r = 0.95$, $P < 0.001$; 31 measurements on 16 individuals). Similar correlations were noted for Ab2 reactivity and thrombocytopenia for homosexuals ($r = 0.90$, $P < 0.001$) and narcotic addicts (r

Table II. Ab2 in HIV-1-ITP Patient Sera

Ab1-F $(ab')_2$	Detection sensitivity of HSITP serum IgG, Ab2										
	E	F	G	H	I	J	K	L	M	N	C1–C5
NITP-A	2960	880	83	440	4440	33	260	210	720	440	0
HSITP-D	3360	1600	110	1320	4440	80	300	170	880	440	0
	Detection sensitivity of NITP serum IgG, Ab2										
	O	P	Q	R	S	T	U	V	W	X	Y
NITP-A	760	280	480	190	1920	1120	110	50	1600	1160	3820
HSITP-D	2080	460	220	480	3040	800	44	50	1280	1760	2400

A solid-phase ^{125}I-labeled protein A assay was used to determine the sensitivity of affinity-purified Ab2 for the detection of $F(ab')_2$ fragments of affinity-purified Ab1 at 200 ng/well. Serum IgG was serially diluted at doubling dilutions for the detection of Ab2, starting at 200 ng/well. The linear portion of the curve was extrapolated to the baseline *x*-axis, which represented control reactivity, e.g., $F(ab')_2$ plus ^{125}I-labeled protein A. The concentration of Ab2 intersecting this baseline represents the detection sensitivity of the assay and is given in picograms/well. C1–C5 refer to five different control IgG's.

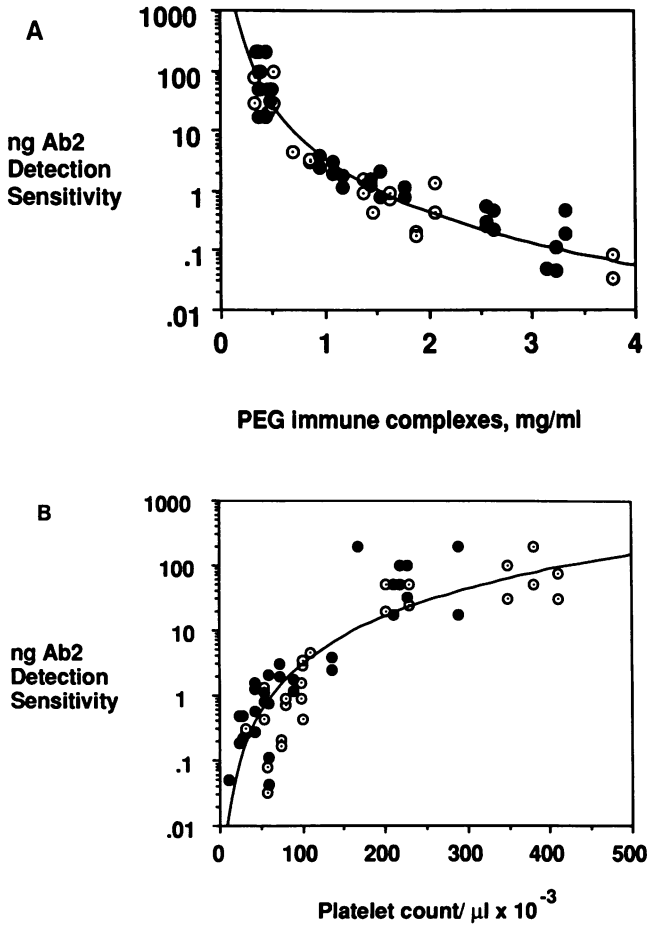

Figure 5. Correlation of Ab2 with (*A*) PEG-precipitable immune complex levels (r = 0.91, $P < 0.001$) and (*B*) platelet count (r = 0.89, $P < 0.001$). The data from 59 paired measurements of HIV-1-seropositive homosexuals (○) and narcotic addicts (●) is presented as a semilog plot. The data include measurements from 10 nonthrombocytopenic HIV-1-seropositive subjects in addition to 21 of the thrombocytopenic patients of Table II. ng Ab2 detection sensitivity refers to the lowest concentration of Ab2 capable of reacting with $F(ab')_2$ fragments of Ab1.

= 0.87, $P < 0.001$). Fig. 5, *A* and *B*, depicts semilog plots of the individual data points for all 59 measurements on 30 HIV-1-seropositive homosexuals and narcotic addicts.

Presence of Ab1 in PEG-precipitable immune complexes. Further experiments were performed to determine whether Ab1 was present in the PEG-precipitable immune complexes of HSITP and NITP patients. This was accomplished with acid elution by a strategy previously described (5). Fig. 6 demonstrates the immunoblots obtained with affinity-purified Ab1 from an HSITP and an NITP patient, respectively, on nitrocellulose strips containing multiple HIV-1 antigens. Note reactivity of Ab1 with a gp120 band (lanes *A2* and *B2*). Note reactivity of the patient's serum with numerous HIV-1 antigens (lane *A1*). (The remainder of the figure is cited below, under Reactivity of Ab1 and Ab2 with gp120 immunoblot.)

Binding of Ab1 and Ab2 derived from sera to platelets. As noted previously (6), affinity-purified Ab1 did not bind to platelets at a concentration as high as 70 µg/ml. Affinity-purified Ab2 also had no reactivity at a concentration of 7 µg/ml. However, the combination of both antibodies (preincubated at 22°C for 2 h) did bind to platelets, whether the antibodies were obtained from the same patient (Fig. 7) or from two different patients (data not shown). Optimum binding to platelets was noted at Ab1/Ab2 ratios of 5:1 and 10:1 with Ab2 held constant and at 10:1 and 20:1 with Ab1 held constant.

Fig. 8 demonstrates binding of affinity-purified Ab1 and Ab2 derived from PEG-precipitable immune complexes to platelets. Preincubation of Ab1 and Ab2 (2 h at 22°C) resulted in an immune complex capable of binding to platelets in a concentration-dependent manner. Ab1 alone or Ab2 alone did not bind to platelets. These results are similar to those noted for affinity-purified Ab1 and Ab2 from sera (Fig. 7).

Reactivity of Ab1 and Ab2 from PEG precipitates with gp120 on immunoblot. To determine whether Ab2 was internal-image type antiidiotype antibody, experiments were performed on immunoblot to determine whether Ab2 would block the binding of Ab1 to gp120. This was not observed. On the contrary, Ab2 and Ab1 intensified the binding of the enzyme-conjugated anti-human indicator antibody to gp120 when both antibodies were added together (Fig. 6, lanes *A7–A9*

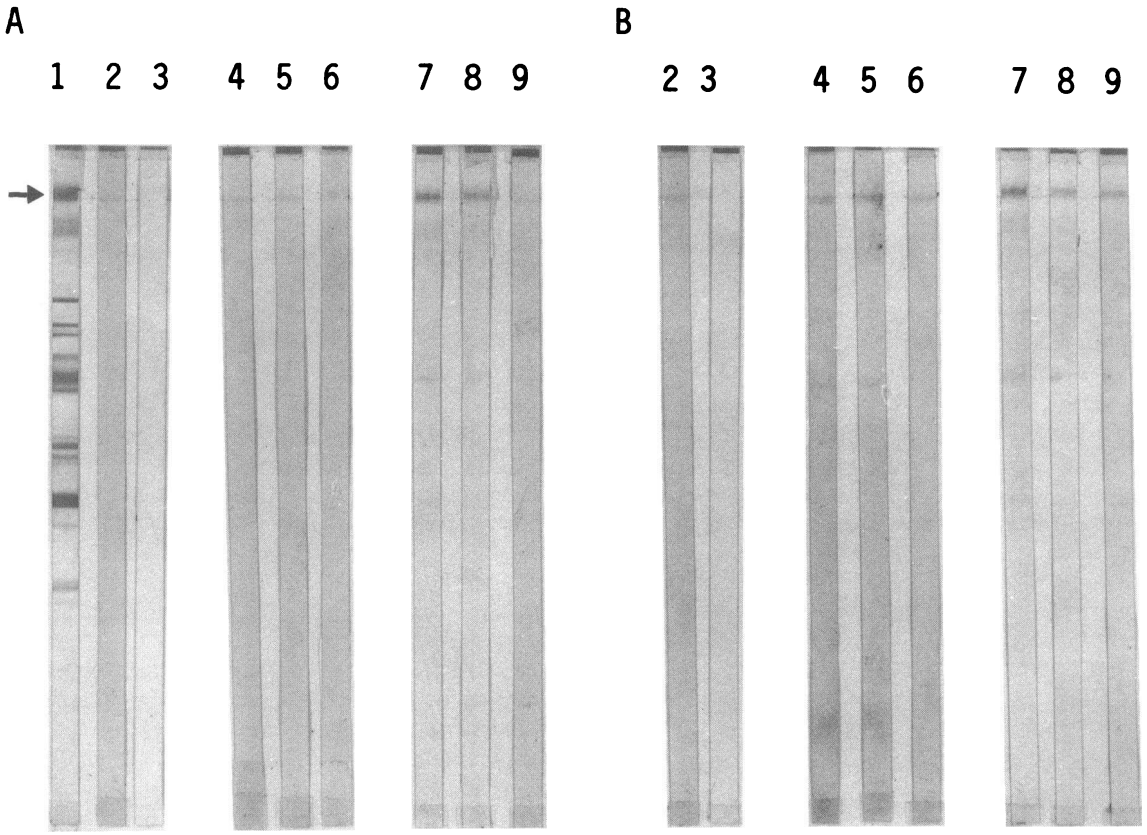

Figure 6. Immunoblot of Ab1 and Ab2 affinity purified from PEG-precipitable immune complexes. Ab1, Ab2, and preincubated Ab1 plus Ab2 were applied to nitrocellulose strips containing the HIV-1 antigens. Reactivity was detected by ELISA with an anti–human IgG antibody coupled to horseradish peroxidase. (*A*) Patient NITP-R. (*B*) Patient HISTP-J. Lane *A1* represents reactivity of HIV-positive sera at a 1:50 dilution; lane *A2,* Ab1 at 0.074 μg/ml; lane A3, Ab2 at 0.74 μg/ml; lanes *A4–A6,* Ab1 at 0.074 μg/ml plus irrelevant IgG at 0.74, 0.38, and 0.19 μg/ml, respectively; lanes *A7–A9,* Ab1 at 0.074 μg/ml plus Ab2 at 0.74, 0.38, and 0.19 μg/ml, respectively. *B* is similar to *A,* except for an Ab1 concentration of 0.27 μg/ml with similar ratios of Ab2 and irrelevant IgG. Arrow refers to gp120 location.

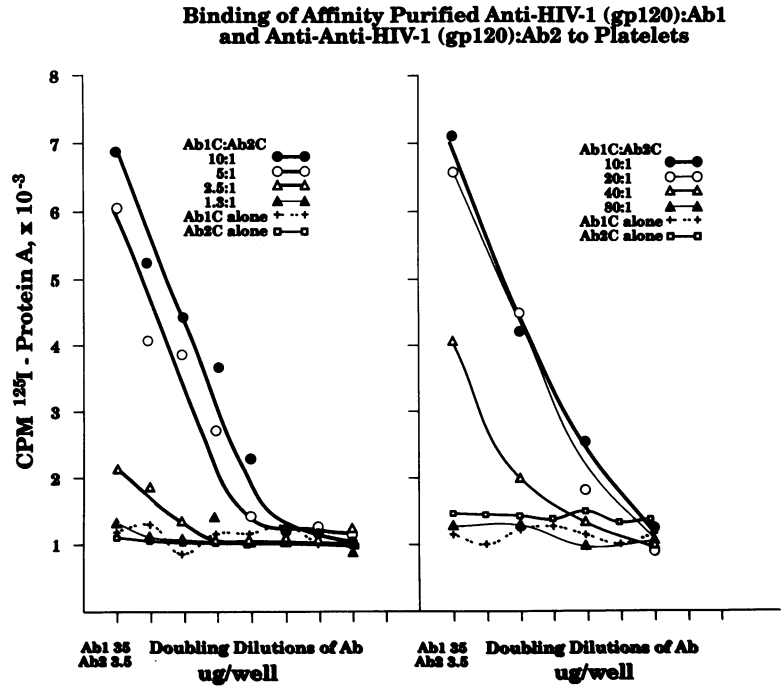

Figure 7. Binding of affinity-purified Ab1 and Ab2 from patient sera to platelets. 10^7 washed platelets were applied to microtiter plates, blocked and washed with 3% Blotto, and then reacted with Ab1, Ab2, or a combination of Ab1 plus Ab2 (preincubated for 2 h at 22°C at various ratios). Non-adherent Ab was washed away and adherent antibody monitored with ^{125}I-labeled staphylococcal protein A. (*Left*) Top curve (●) represents Ab1/Ab2 at a protein ratio of 10:1 (i.e., 35:3.5 μg/well). The bottom curves represent Ab2 held constant at 3.5 μg/well and Ab1 diluted to result in ratios of 5:1 (○), 2.5:1 (△), and 1.3:1 (▲) before doubling dilutions of the complex onto platelets. (*Right*) Ab1 was held constant at 35 μg/well and Ab2 diluted to result in ratios of 20:1 (○), 40:1 (△), and 80:1 (▲).

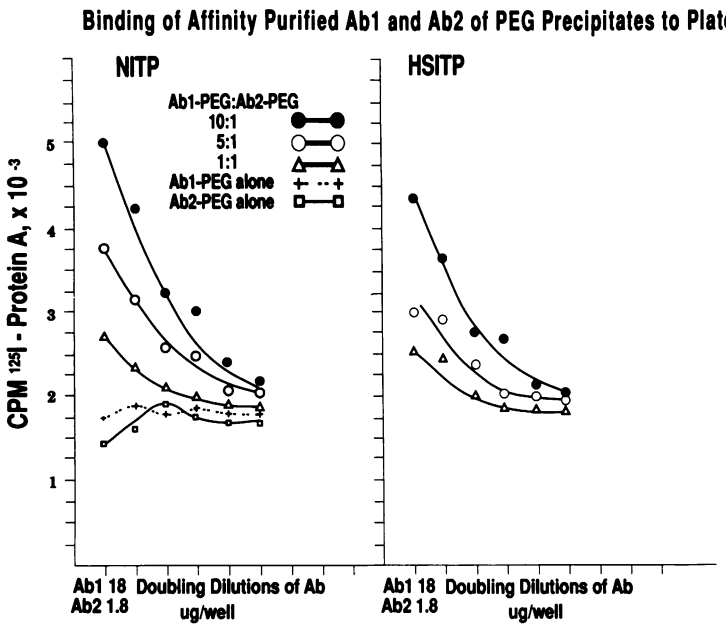

Figure 8. Binding of affinity-purified Ab1 and Ab2 of PEG-precipitable immune complexes to platelets of patients NITP-R and HSITP-J. Ab1 and Ab2 and their combination (after preincubation) were reacted with washed platelets on a microtiter plate as in Fig. 7. Ab1 was held constant. The ratio of Ab1/Ab2 was 10:1 (i.e., 18:1.8 μg/well) (●), 5:1 (○), and 1:1 (△), before doubling dilutions of the complex onto platelets.

and *B7–B9*). Compare to control lanes *A4–A6* and *B4–B6* in which Ab1 was added together with irrelevant IgG.

Discussion

PEG-precipitable serum protein is three- to sevenfold greater in HIV-1-ITP patients than in either normal subjects or classic ATP patients (2, 4). Platelet-bound IgG and C3C4 are three- to fourfold greater and platelet-bound IgM two- to threefold greater than in normal subjects (2, 4). It has recently been shown that platelets of HIV-1-ITP patients contain elutable Ab1 in the absence of HIV-1 antigen and that the Ab1 is present as a high-molecular-weight complex (6). In addition, the sera of these patients contain public anti-F(ab')$_2$ antibodies (5). It has been suggested, via qualitative studies on immunoblot, that some of these anti-F(ab')$_2$ antibodies may be antiidiotypes against Ab1 (6). Thus, an attractive hypothesis for the marked deposition of Ig and C on these platelets is the deposition of immune complexes containing Ab1–Ab2 complexes, presumably present in the PEG-precipitable material, onto platelets; thrombocytopenia could result at least in part from phagocytosis of opsonized platelets by monocytes and fixed macrophages. Alternatively, or in addition, there is evidence that thrombopoiesis can be impaired (12–16), possibly via direct invasion of megakaryocytes with HIV-1 virus (17, 18) or possibly via immune complex–mediated megakaryocyte damage.

In this report, we have carefully examined the PEG-precipitable protein in HIV-1-ITP patients for its Ig composition, Ab1 reactivity, and ability to bind to platelets. Gel filtration studies demonstrate that > 90% of the precipitable protein is present as a high-molecular-weight complex containing Ab1 ruling out the possibility that the elevated values of PEG-precipitable complexes in these patients are due to precipitation of 7S IgG in hypergammaglobulinemic patients. The Ab1 reactivity of the high-molecular-weight complex binds to platelets in a saturation-dependent manner. This high-molecular-weight complex contains IgG, IgM, C3, and Ab1 reactivity in the absence of HIV-1 antigen (6). Affinity chromatography with protein A, anti-IgM, and anti-C3 columns reveals that ~ 95% of the Ab1 reactivity in the PEG-precipitable protein is present as a macromolecular complex of IgG, IgM, and C3. Almost all of the IgG, 86–96% of the C3, and 37–49% of the IgM binds to platelets. Thus, some of the IgM contains Ab1 reactivity and does not bind to platelets, possibly because it is not present as a macromolecular complex.

Because of the suggestion from qualitative immunoblot studies (6) that Ab1 might be present as an Ab1–Ab2 complex in HIV-1-ITP patients, their sera and PEG-precipitable immune complexes were examined for Ab2 by use of a highly sensitive quantitative immunoassay. Ab1 was affinity purified with recombinant gp120 and Ab2 was affinity purified with Ab1. 21 consecutive HIV-1-ITP patients with elevated PEG-precipitable immune complexes (10 HSITP and 11 NITP patients) had detectable Ab2 in their sera, whereas 10 consecutive nonthrombocytopenic HIV-1–seropositive subjects had barely detectable levels and 5 consecutive control subjects had no detectable Ab2. In addition, Ab2 could be eluted from their PEG-precipitable immune complexes, and there is a significant correlation of Ab2 reactivity with circulating immune complex level. A similar correlation has been reported for nonspecific anti-F(ab')$_2$ antibodies in these patients (5). Ab1 could also be eluted from their PEG-precipitable immune complexes. Thus anti-F(ab')$_2$ antiidiotype-like HIV-1 Ab complexes circulate in the sera of HIV-1-ITP patients and deposit on their platelets.

Since Ab2 did not block the binding of Ab1 to its epitope, one should consider the possibility that Ab2 may be directed against the framework region of Fab rather than acting as a true antiidiotypic Ab. In that case, the lack of reactivity of Ab2 with F(ab')$_2$ from control preparations would suggest that Ab1 in these patients tends to use an uncommon VH and/or VK region, not much represented in normal IgG. The greater reactivity of Ab2 for autologous than for homologous F(ab')$_2$ Ab1 could then be explained on the basis of somatic mutations unique to the Ab1 of that individual. Indeed, although Ab2 reacted with Ab1 from different patients, its reactivity with autologous Ab1 was 4–120-fold greater than its reactivity with

homologous Ab1. Thus, the patients demonstrated greater auto- than cross-reactive Ab2 reactivity. These data indicate that the Ab2 produced by a patient are directed against autologous Ab1 idiotypes as well as against idiotypes shared by different patients, producing antibodies against similar or identical gp120 epitopes.

Regardless of the exact specificity, it is evident that Ab1 and Ab2 are responsible for immune complex formation and deposition on platelets. Evidence for deposition of immune complexes on platelets was obtained in vitro by the demonstration that (*a*) PEG-precipitable complexes bind to platelets in a saturation-dependent manner and (*b*) affinity-purified Ab1 and Ab2 do not bind to platelets independently but do bind as a complex in a saturation-dependent manner and ex vivo by the demonstration that Ab1 and Ab2 can be eluted from platelets and Ab2 shown to react on immunoblot with Ab1 (6).

The ability of PEG-precipitable immune complexes as well as affinity-purified Ab1-Ab2 complexes to bind to platelets in vitro, and the ex vivo presence of these complexes on platelets (6), suggests that they may be responsible for or contribute to the markedly elevated Ig content of these platelets. The likely presence of free Fc and C3 domains in the macromolecular complexes suggests that they may be cleared by the patient's reticuloendothelial system via Fc and C3b receptors. This suggestion is supported by the significant positive correlation between Ab2 reactivity (i.e., idiotype antiidiotype formation) and thrombocytopenia and by the clinical response of HIV-1-ITP patients to corticosteroids, splenectomy, or intravenous gamma globulin—agents or procedures that block reticuloendothelial clearance. The ability of immune complexes to induce thrombocytopenia is supported by experimental studies in rabbits, wherein thrombocytopenia was induced by immune complex infusion (19, 20), as well as by a clinical study of thrombocytopenia in patients with classic immune complex disease (SLE), which demonstrated the presence of pepsin-sensitive immune complexes in their sera that were capable of binding to normal platelets in the absence of pepsin digestion (21).

Thus, HIV-1-ITP patients have circulating anti-idiotype-like HIV-1 antibody complexes that are present in a PEG-precipitable macromolecular complex and capable of binding to platelets in a saturation-dependent manner. It is likely that this is responsible, at least in part, for the markedly elevated platelet Ig content of their platelets. We postulate that this may be responsible for their peripheral platelet destruction.

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