Antiidiotype Antibody against Platelet Anti-GPIIIa Contributes to the Regulation of Thrombocytopenia in HIV-1–ITP Patients

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Abstract

Patients with human immunodeficiency virus 1–associated immunological thrombocytopenia (HIV-1–ITP) have markedly elevated platelet-bound immunoglobulin (Ig)G, IgM, and C3C4, as well as serum circulating immune complexes (CICs) composed of the same. Affinity purification of IgGs from their CICs with fixed platelets reveals high-affinity antibody (Ab) against platelet glycoprotein (GP)IIIa 49–66, which correlates inversely with their platelet count. However, sera from these patients have little to no anti-GPIIIa activity. To investigate this, we assayed serum, purified serum IgG, and CIC-Ig from these patients. This revealed ~150-fold greater Ab activity in purified serum IgG, and ~4,000-fold greater reactivity in CIC-IgG. This was shown to be associated with the presence of antiidiotype Ab2 (both IgG and IgM) sequestered in the CIC-IgG. The IgM antiidiotype was predominantly blocking Ab, as demonstrated by specificity for $F(ab')_2$ fragments of anti–GPIIIa 49–66 of HIV-1–ITP patients and inhibition of reactivity with peptide GPIIIa 49–66, not with a control peptide. The IgM antiidiotype was not polyreactive. Similar measurements were made in nonthrombocytopenic HIV-1–infected patients. Their serum reactivity was not measurable, but serum Ig and CIC-IgG against platelet GPIIIa 49–66 was present, although considerably lower than that found in HIV-1–ITP patients (26- and 35-fold lower, respectively). In addition, their IgM antiidiotype reactivity was 12-fold greater than that found in HIV-1–ITP patients. The IgM antiidiotype Ab titer of both cohorts correlated with in vivo platelet count ($r = 0.7$, $P = 0.0001$, $n = 32$). To test the in vivo effectiveness of the IgM antiidiotype, thrombocytopenia was induced in mice with 25 μg of affinity-purified anti–GPIIIa 49–66 (mouse GPIIIa has 83% homology with human GPIIIa and Fc receptors for human IgG1). Maximum effect was obtained at 4–6 h after intraperitoneal injection into Balb/c mice with a platelet count of ~30% baseline value. Preincubation of the anti-GPIIIa Ab with control IgM at molar ratios of IgM/IgG of 1:7 before intraperitoneal injection had no effect on the in vivo platelet count, whereas preincubation with patient IgM antiidiotype improved the platelet count to 50–80% of normal. Thrombocytopenia could be reversed after addition of IgM antiidiotype 4 h after induction of thrombocytopenia. Thus, CICs of HIV-1–infected patients contain IgM antiidiotype Ab against anti-GPIIIa, which appears to regulate their serum reactivity in vitro and their level of thrombocytopenia in vivo.

Key words: platelet • HIV • autoimmunity • antiidiotype antibody • AIDS

Introduction

Immunological thrombocytopenia is a common complication of HIV-1 infection. The incidence in HIV-1–infected patients of HIV-1–associated immunological thrombocytopenia (HIV-1-ITP)[1] is 0–21% at onset and increases to 30% or more with the development of AIDS (1, 2). Kinetic data on platelet survival strongly suggest that early-onset HIV-1–ITP is secondary to increased peripheral destruction of platelets, whereas patients with AIDS are more likely to have decreased platelet production (3). Patients

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[1]*Abbreviations used in this paper:* ATP, autoimmune thrombocytopenia; CIC, circulating immune complex; GP, platelet glycoprotein; HIV-1–ITP, immunological thrombocytopenia associated with HIV-1–infection; IC, immune complex.

with early-onset HIV-1–ITP have a thrombocytopenic disorder that is clinically indistinguishable from classic autoimmune thrombocytopenia (ATP), seen predominantly in females (4–7). However, HIV-1–ITP is different from classic ATP with respect to the predominant male incidence and the markedly elevated platelet-associated IgG, IgM, and C3C4, as well as presence of circulating serum immune complexes (CICs) composed of the same (5, 6). These complexes contain anti-$F(ab')_2$ Abs (8) as well as HIV-1–related Abs (9, 10). Affinity purification of IgGs from their CICs with platelets has revealed high-affinity IgG1 Ab against the platelet integrin glycoprotein (GP)IIIa peptide 49–66 (11, 12). This serum anti-GPIIIa Ab correlates inversely with platelet count ($r = 0.71$; reference 12) and induces severe thrombocytopenia in mice (12), which can be prevented and/or reversed with GPIIIa 49–66 peptide (reference 12; mouse GPIIIa is 83% homologous with human GPIIIa, and macrophages have Fc receptors for human IgG1).

However, we have recently observed that sera from HIV-1–ITP patients have considerably less anti–GPIIIa 49–66 reactivity compared with ~150-fold greater reactivity in purified IgG from serum and ~4,000-fold greater reactivity sequestered in their serum CICs. This suggested the possibility of blocking or antiidiotype Ab against anti-GPIIIa in these patients.

This report documents the presence of blocking IgM antiidiotype antibody (Ab2β and/or Ab2γ) versus anti–GPIIIa 49–66 in these patients, which correlates with their platelet count ($r = 0.7$, $P = 0.001$, $n = 32$) and reverses in vivo induced thrombocytopenia in mice.

Materials and Methods

Population. The population consists of 37 early-onset HIV-1–infected patients without AIDS (19 homosexuals and 18 drug abusers). 22 were thrombocytopenic, and 15 had normal platelet counts. Five control sera were obtained from healthy laboratory personnel. Seven sera were obtained from classic ATP patients.

Purified IgG. IgG was prepared from serum by ion-exchange chromatography (13).

$F(ab')_2$. $F(ab')_2$ fragments were prepared from purified IgG by pepsin digestion as described (13), and were shown to be free of Fc fragments by SDS-PAGE as well as ELISA (13).

Immune Complexes. Immune complexes (ICs) were prepared from serum by polyethylene glycol precipitation (5). Precipitates were dissolved in one fifth of their serum volume in 0.01 M PBS, pH 7.4.

Isolation of IgG and IgM from ICs. IgG and IgM were isolated and purified as described (11). In brief, polyethylene glycol (PEG)-ICs were applied to a staphylococcal protein A affinity column (Sigma-Aldrich). The bound complex was washed with PBS and eluted with 0.1 M glycine buffer, pH 2.5. The eluted material was applied to an acidified sephadex G-200 gel filtration column (Amersham Pharmacia Biotech) preequilibrated with the same elution buffer. Effluents of the IgG peak were isolated, neutralized, dialyzed against PBS, and applied to a rabbit anti-IgM affinity column (ICN Pharmaceuticals, Inc.) prepared from Affi-Gel 10 (Bio-Rad). The flow-through material was free of contaminating IgM by immunoblot and ELISA. Effluents of the IgM peak were isolated, neutralized, dialyzed against PBS, and applied to an anti–Fc receptor affinity column to remove rheumatoid factor. (Fc fragments were prepared by papain digestion [11] and affinity purified on a staphylococcal protein A column; the acid eluate was verified by SDS-PAGE and was coupled to Affi-Gel 10). The flow-through IgM was devoid of rheumatoid factor, as determined by inability to bind to a second Fc column.

Affinity Purification of Antiplatelet IgG. Antiplatelet IgG was affinity purified with 10^8 platelets fixed with 2% paraformaldehyde for 2 h at room temperature, followed by overnight gentle rocking at 4°C, then acid elution and neutralization, as described (11). The IgG subclass, determined by radial immunodiffusion (The Binding Site), was IgG1 with both k and l light chains.

Affinity Purification of Antiplatelet GPIIIa 49–66. Peptide GPIIIa 49–66, CAPESIEFPVSEARVLED (synthesized by Quality Controlled Biochemicals), was coupled to an affinity column with the heterobifunctional cross-linker sulfo-succinimidyl 4-(*N*-maleimidomethyl) cyclohexane-1-carboxylate as recommended by the manufacturer (Pierce Chemical Co.; cross-links the resin with NH_2-terminal cysteine of the peptide), and was incubated with 0.4 ml of affinity-purified IgG overnight at 4°C. The column was then washed, eluted at pH 2.5, and neutralized as described (12).

ELISA. Antibody reactivity was measured by solid-phase ELISA (12, 13) using serial doubling dilutions of IgG or IgM on U-shaped polyvinyl microtitre plates (Curtin-Matheson Scientific) preincubated overnight at 4°C with 200 ng of peptide GPIIIa 49–66 or $F(ab')_2$ fragment of anti–GPIIIa 49–66 in PBS, and was blocked with 2.5% BSA in PBS. A minimum of two different $F(ab')_2$ fragments were used for each experiment. The first Ab, used to detect IgG binding, was a 1:500 dilution of goat $F(ab')_2$ anti–human IgG (γ chain specific) coupled to alkaline phosphatase (Sigma-Aldrich). The second Ab, used to detect IgM binding, was a 1:1,000 dilution of goat $F(ab')_2$ anti–human IgM (μ chain specific) coupled to alkaline phosphatase (ICN Pharmaceuticals, Inc.). Appropriate enzyme substrate was added, and color was read in an automated micotitre plate reader at 405 nm. In some experiments, bound anti–GPIIIa 49–66 was preincubated with GPIIIa 49–66 peptide for 2 h at room temperature before testing for antiidiotype Ab binding.

IgM Ab Titer. IgM Ab titer refers to the reciprocal of the lowest concentration of IgM Ab (μg/well) capable of binding to its antigen, determined by extrapolation of the linear portion of the binding curve to zero binding.

Induction of Thrombocytopenia in Mice with Anti–GPIIIa 49–66. Human affinity-purified anti–GPIIIa 49–66 (25 μg) was injected intraperitoneally into BALB/c mice (Taconic Farms), and blood was drawn from the retroorbital sinus at various times. In some experiments, anti–GPIIIa 49–66 Ab was preincubated with either control IgM or antiidiotype IgM before intraperitoneal injection; in other experiments, control or antiidiotype IgM was given after 4 h of thrombocytopenia. Platelet counts were determined from 20 μl of blood drawn into Unopettes (no. 5855; Becton Dickinson) containing optimal anticoagulant concentration and diluent for quantitating platelet count by phase microscopy.

Results

Fig. 1 demonstrates a comparison of the relative binding reactivity of serum, serum IgG, and purified IC-IgG for peptide GPIIIa 49–66 in a representative experiment of

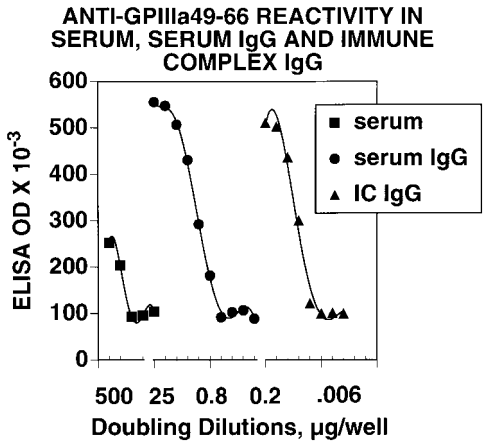

Figure 1. Antiplatelet GPIIIa 49–66 reactivity of serum, serum IgG, and IC-IgG of one representative HIV-1–ITP patient of five patients studied. Various IgG concentrations of serum, purified serum IgG, and purified IC-IgG were assayed for anti–GPIIIa 49–66 reactivity by solid-phase ELISA as described in Materials and Methods.

five different patients (Table I, HIV-1–ITP). 50% detection sensitivity for the respective Ab cohorts were ~125, 0.8, and 0.03 μg/well. Thus, serum IgG has ~150-fold greater reactivity than serum, and IC-IgG has approximately sevenfold greater reactivity than serum IgG (~4,000-fold greater than serum). Similar studies performed on sera of nonthrombocytopenic HIV-1–infected patients also detected antiplatelet GPIIIa 49–66 in their serum IgG and CIC-IgG, but at considerably lower levels (25- and 35-fold less, respectively; Table I; HIV-1 controls). This suggested the possibility of blocking or antiidiotype Ab in serum.

In contradistinction to HIV-1–ITP patients, minimal to absent serum antiplatelet reactivity was noted in seven classic ATP patients, with no enhancement of reactivity noted with serum IgG or IC-IgG (data not shown).

Presence of IgG Antiidiotype Ab versus Anti–GPIIIa 49–66. Fig. 2 A demonstrates binding of purified IC-IgG Ab from five different HIV-1–ITP patients to $F(ab')_2$ fragments of affinity-purified anti–GPIIIa 49–66. 50% binding was observed at ~2 μg/ml. Similar results were obtained with a second $F(ab')_2$ fragment (data not shown). No binding was obtained with the same five IC-IgG preparations against two different control $F(ab')_2$ fragments (one of which is shown in Fig. 2). No binding was obtained with five different control IC-IgG preparations (data not shown).

Fig. 3 A demonstrates poor to partial blocking of binding of IgG antiidiotype Ab to anti–GPIIIa 49–66 with peptide GPIIIa 49–66. Thus, only 20% of Ab binding could be inhibited at a peptide/$F(ab')_2$ molar ratio of 1,024:1, and therefore designated blocking ($P < 0.05$ for last three concentrations of peptide; one-tail Student's *t* test).

Presence of IgM Antiidiotype Ab versus Anti–GPIIIa 49–66. Fig. 2 B demonstrates binding of IgM Ab to anti–GPIIIa 49–66. 50% binding was observed at ~1.25 μg/ml. Similar results were obtained with a second $F(ab')_2$ fragment (data not shown). IgM antiidiotype specificity was next examined against five different control $F(ab')_2$ fragments and four different antigens to determine whether this IgM could represent polyclonal germline IgM secreted by

Table I. *Clinical and Immunological Data on HIV-1–ITP and HIV-1 Control Patients*

						anti–GPIIIa 49–46 50% Binding			
	Patient	Age	Sex	Hx	PC	Serum	IgG	IC-IgG	Reciprocal titer IgM Ab2β versus anti–GPIIIa 49–66
							μg/well		
HIV-1–ITP	2	34	M	HS	114	62	0.156	0.025	354
	21	40	M	HS	87	125	0.156	0.025	690
	5	26	M	IVDA	52	125	0.156	0.050	930
	3	38	M	HS	40	125	3.13	0.025	420
	20	33	F	IVDA	31	62	3.13	0.025	154
						log mean:	1.1	0.029	430
HIV-1 controls	49	29	M	IVDA	261	NM	32	0.6	2,128
	54	35	M	HS	257	NM	32	0.6	6,666
	47	36	M	IVDA	187	NM	16	1.3	8,333
	48	43	M	IVDA	173	NM	32	1.3	6,666
	53	37	M	HS	164	NM	32	1.3	4,762
						log mean:	28	1.0	5,188

Hx, history; HS, homosexual; IVDA, intravenous drug abuser; PC, platelet count/μl; NM, not measurable.

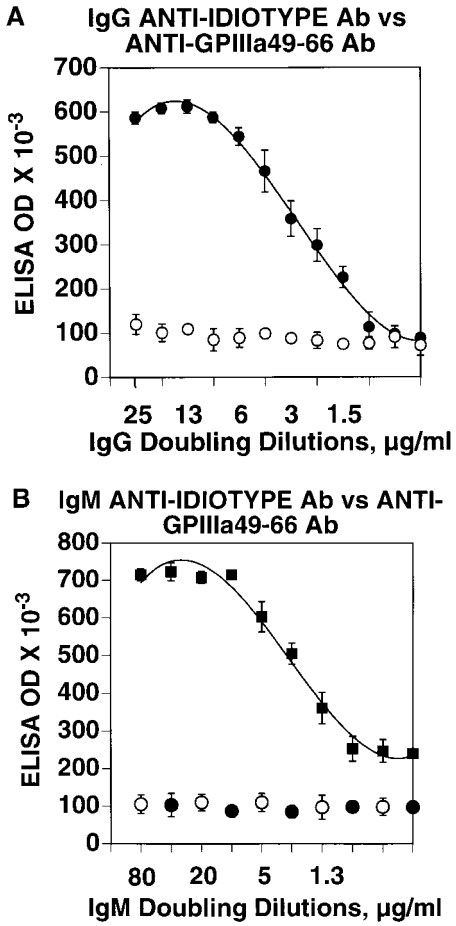

Figure 2. IgG and IgM antiidiotype reactivity against $F(ab')_2$ fragments of affinity-purified antiplatelet GPIIIa 49–66. (A) IgG antiidiotype. Purified IC-IgG was reacted with anti–GPIIIa 49–66 by solid-phase ELISA. The mean ± SEM of five IC-IgG experiments (●) is given. Similar results were obtained with a second anti–GPIIIa 49–66 $F(ab')_2$ fragment. The same five IC-IgG samples were nonreactive with four different control $F(ab')_2$ fragments, one of which is shown (○). The blank rate without second detecting Ab varied between 0.025–0.100 OD units. (B) IgM antiidiotype. Purified IC-IgM was reacted with anti–GPIIIa 49–66. The mean ± SEM of five experiments (■) is given. Similar results were obtained with a second anti–GPIIIa 49-66 $F(ab')_2$ fragment. No reactivity was noted with 16 IC-IgM preparations against five different control $F(ab')_2$ preparations, one of which is shown (○). No reactivity was noted with five control IgM preparations against two patient $F(ab')_2$ fragments, one of which is shown (●). The blank rate without second detecting Ab varied from 0.130–0.145 OD units.

$CD5^+$ B1 cells (14). No binding was obtained with 16 positively reacting IC-IgM preparations against five different control $F(ab')_2$ fragments. One of these experiments is shown in Fig. 2 B. No binding to anti–GPIIIa 49–66 was obtained with five different control IgM preparations made from control subject IC-IgM (Fig. 2 B). No binding was obtained with four different proteins: ovalbumin, soybean trypsin inhibitor, thyroglobulin, or carbonic anhydrase (Fig. 4).

Fig. 3 B demonstrates considerable blocking of binding of IgM antiidiotype Ab to anti–GPIIIa 49–66 with peptide GPIIIa 49–66. Thus, 50% of Ab binding could be inhibited at a peptide/$F(ab')_2$ molar ratio of ~1:6.4, and therefore be designated blocking antibody (Ab2β and/or Ab2γ). No blocking was noted with irrelevant peptide CGYGP-KKKRKVGG at a peptide/$F(ab')_2$ ratio of 1,024:1. IgM antiidiotype Ab did not bind to peptide GPIIIa 49–66 (five experiments, data not shown).

Fig. 3 C demonstrates blocking of binding of anti–GPIIIa 49–66 to platelets by IgM antiidiotype, not by control IgM or IgG antiidiotype. Thus, 50% of Ab binding to platelets could be inhibited at an IgM/IgG molar ratio of ~1:10.

Correlation between IgM Antiidiotype Ab versus Anti–GPIIIa 49–66 and Patient's Platelet Count. The above data suggested that the reason for the relatively weak serum anti–GPIIIa 49–66 reactivity with its Ag was because of the presence of IgM blocking antiidiotype Ab in its serum. We therefore reasoned that if this were true and pathophysiologically relevant, then a positive correlation should be obtained between the antiidiotype titer and the patient's platelet count. This proved to be the case. Fig. 5 demonstrates such a correlation of $r = 0.71$ ($P = 0.001$, $n = 32$). Specific measurements and clinical data are described in Table I, lanes 1–9. In these two cohorts, IgM antiidiotype Ab was 12-fold greater in nonthrombocytopenic patients. A similar log mean IgM antiidiotype Ab titer of 1:3,643 was found in 10 additional nonthrombocytopenic HIV-1 patients.

IgM Antiidiotype versus Anti–GPIIIa 49–66 Reverses In Vivo Thrombocytopenia Induced in Mice by Anti–GPIIIa 49–66. Our previous study demonstrated that human anti–GPIIIa 49–66 Ab could induce significant thrombocytopenia in mice, with nadir at 4 h when injected intraperitoneally (control IgG had no effect; reference 12). We therefore tested the ability of the IgM antiidiotype Ab versus anti–GPIIIa 49–66 to reverse this effect in vivo. Again, this proved to be the case. Thus, Fig. 6 A demonstrates a 70% drop in platelet count induced by 25 μg/ml of anti–GPIIIa 49–66, with reversal to 50–80% of normal by preincubation with IgM antiidiotype/anti–GPIIIa 49–66 ratios of 1:7, respectively. Neither control IgM nor IgG-antiidiotype, at similar ratios, had any effect. To rule out the possibility that the IgM antiidiotype Ab was not operating through increased clearance of the antiplatelet Ab, experiments were also performed after induction of the thrombocytopenia at 4 h. Fig. 6 B demonstrates reversal of thrombocytopenia with the IgM antiidiotype Ab, not with the IgG antiidiotype, thus confirming that the IgM antiidiotype was interfering with the binding of anti-GPIIIa to platelets.

Discussion

These data clearly indicate the presence of IgG and IgM antiidiotype (Ab2) against anti–GPIIIa 49–66 in HIV-1–

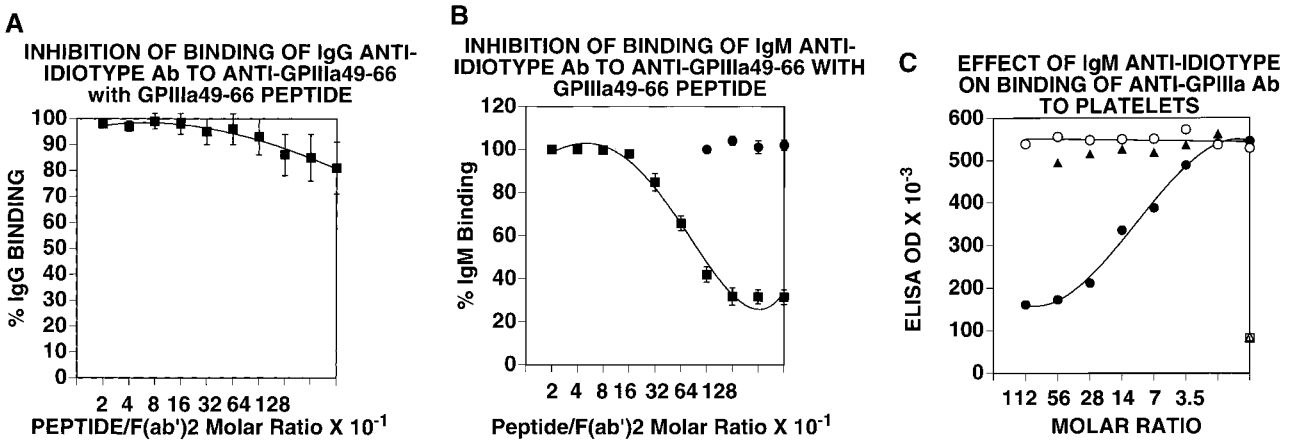

Figure 3. Presence of IgG and IgM antiidiotype reactivity against anti–GPIIIa 49–66. (A) IgG antiidiotype. Various molar ratios of peptide/$F(ab')_2$ anti–GPIIIa 49–66 fragments were preincubated as described in Materials and Methods before addition of antiidiotype Ab. The mean is given for five experiments. (B) IgM antiidiotype. Various molar ratios of peptide/$F(ab')_2$ fragments were preincubated before addition of the IgM antiidiotype. The mean is given for five experiments (■). The mean of five additional experiments with irrelevant peptide is also given (●). (C) Inhibition of anti–GPIIIa 49–66 binding to platelets with IgM antiidiotype. Binding of anti–GPIIIa 49–66 was measured by solid-phase ELISA, using goat $F(ab')_2$ anti–human IgG coupled to alkaline phosphatase (11). Patient IC-IgM (●) or control IgM (○) or patient IC-IgG (▲) was preincubated with affinity-purified anti–GPIIIa 49–66 at various molar ratios (IgM/IgG or IgG/IgG) for 2 h at room temperature, then was applied to platelets bound to the microtitre plate, blocked, and washed with 5% BSA-PBS. Symbols in the lower corner of the right vertical line refer to various control measurements: platelets plus IC-IgM plus detection Ab (△), blocked plates plus anti–GPIIIa 49-66 plus IC-IgM plus detection Ab (+), and platelets plus detection Ab (□). Representative of four different IgM antiidiotype preparations.

ITP patients. Their absence in classic ATP patients suggests that the mechanism of thrombocytopenia is different in both autoimmune disorders. The antiidiotype in HIV-1–ITP patients was both Ab2α as well as blocking (Ab2β and/or Ab2γ) for both isotypes, with blocking IgM Ab2 predominating over IgG Ab2. Several lines of evidence support the conclusion that blocking IgM Ab2 is responsible for the impaired serum reactivity of anti–GPIIIa 49–66: (a) purification of serum IgG and IC-IgG increased anti-GPIIIa reactivity ~150- and 4,000-fold, respectively; (b) IgM purified from PEG-IC bound to anti–GPIIIa 49–66 in a specific manner (no binding with control IgM); (c) IgM Ab could be blocked from binding to anti–GPIIIa 49–66 with the Ag for anti–GPIIIa 49-66 (not with an irrelevant Ag); (d) anti–GPIIIa 49–66 could be blocked from binding to platelets with IgM antiidiotype, not with control IgM or IgG antiidiotype; and (e) in vivo thrombocytopenia induced in mice with anti–GPIIIa 49–66 could be reversed with purified IC-IgM, not with purified serum IgM or IC-IgG.

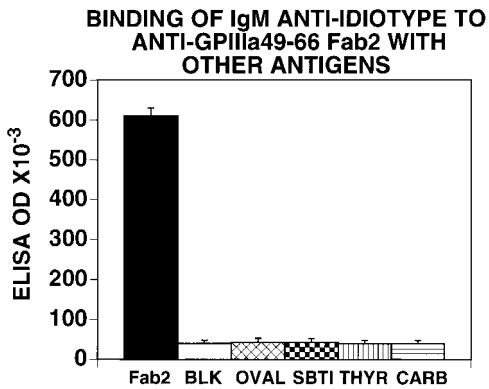

Figure 4. Specificity of IgM antiidiotype Ab for anti–GPIIIa 49–66. Four unrelated antigens, ovalbumin (OVAL), soybean trypsin inhibitor (SBTI), thyroglobulin (THYR), and carbonic anhydrase (CARB), were applied to a microtitre plate in addition to the $F(ab')_2$ fragment of anti–GPIIIa 49–66 and reacted with the IgM antiidiotype Ab versus anti–GPIIIa 49–66. OD refers to the highest value obtained in the linear range of the dilution curve. Blank (BLK) refers to reactivity without the primary Ab. The mean of five experiments is given.

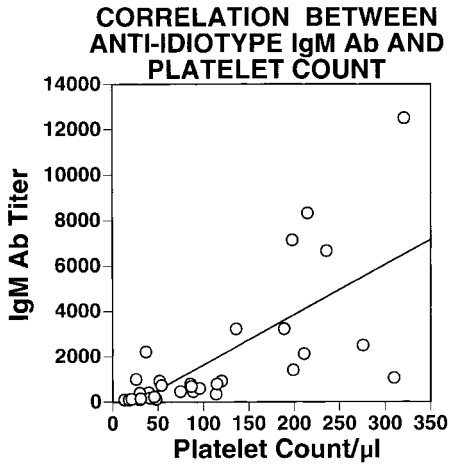

Figure 5. Correlation between HIV-1–ITP patient's platelet count in vivo with IgM antiidiotype titer in vitro. IgM Ab titer refers to the reciprocal of the lowest concentration (µg/well) of IgM antiidiotype reactivity with anti–GPIIIa 49–66, obtained by extrapolation of the linear Ab dilution curve to the x-axis.

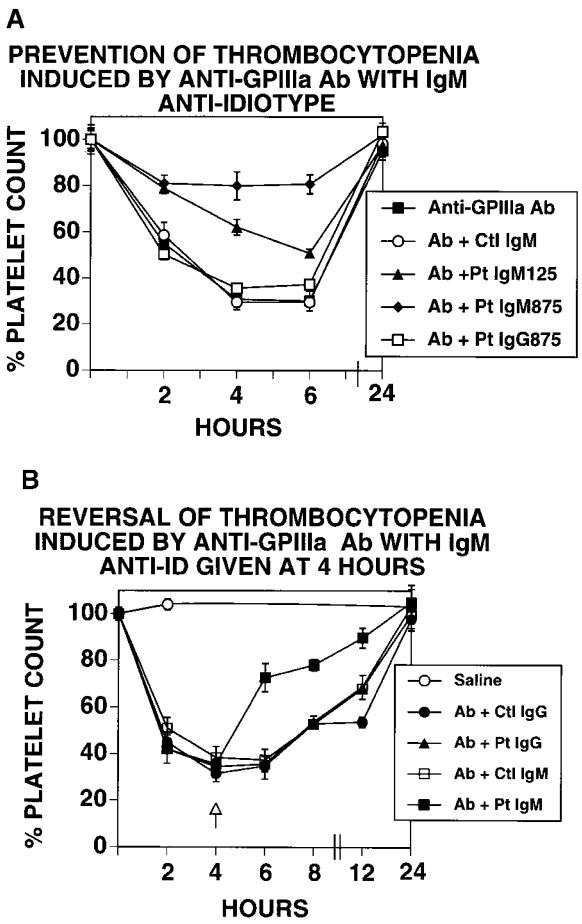

Figure 6. Prevention and reversal of in vivo thrombocytopenia induced by anti–GPIIIa 49–66 induced in mice with IgM antiidiotype Ab against anti–GPIIIa 49–66. (A) Prevention. Four to seven mice in each group were treated intraperitoneally with either anti–GPIIIa 49–66 Ab, Ab plus control IgM (125 μg), Ab plus antiidiotype IgM (125 μg), Ab plus antiidiotype IgM (875 μg), or Ab plus antiidiotype IgG (875 μg). Platelet counts were obtained at various time intervals for 24 h. The mean platelet count ± SEM for all four groups was similar ($1.72 \pm 0.56 \times 10^6$/μl). Pt, patient. (B) Reversal. 16 mice were treated intraperitoneally with 25 μg of anti–GPIIIa 49–66, and 4 mice were treated intraperitoneally with saline. At 4 h, after induction of thrombocytopenia, the 16 antibody-treated mice were treated (4 mice in each group) with 875 μg of either control IgG, patient (Pt) antiidiotype IgG, control IgM, or patient antiidiotype IgM, and were followed for 24 h. Note reversal of thrombocytopenia with IgM antiidiotype at 6, 8, and 12 h compared with control IgM ($P = 0.0008$, 0.0015, and 0.0137, respectively; paired Student's *t* test), not with IgG antiidiotype or control IgG.

These data strongly suggest that the IgM antiidiotype as well as level of anti–GPIIIa 49–66 IgG Ab play a role in regulating early-onset autoimmune HIV-1–ITP in vivo. A correlation of platelet count with IgM antiidiotype of $r = 0.7$ supports this suggestion, as do our previous observations on the presence of an inverse correlation between anti–GPIIIa 49–66 antibody and platelet count in early-onset HIV-1 infection (thrombocytopenic versus nonthrombocytopenic; reference 12). It should be recognized that multiple factors are likely to regulate the platelet count in HIV-1–ITP patients. These include platelet production, platelet survival, and relative phagocytic function of the reticuloendothelial system. It is therefore not surprising that the correlation coefficient is <1.

The presence of anti–GPIIIa 49–66 Ab in nonthrombocytopenic patients, albeit at 26–35-fold lower reactivity than in HIV-1–ITP patients, is of interest and suggests that low levels of reactivity may be present in most early-onset HIV-1–infected patients. It is possible that this may represent molecular mimicry between anti–HIV-1 Abs and platelet GPIIIa. Indeed, this has been reported for anti–HIV-1–gp120 Ab (15).

Our findings on the presence of blocking IgM antiidiotype in HIV-1–ITP patients is reminiscent of previous observations on IgM Abs blocking natural polyreactive low-affinity Abs in mice as well as humans (16–19). However, our anti–GPIIIa 49–66 autoantibody preparation is different, in that it is highly specific (11) and contains high-affinity Ab (Kd = 1–2 nM; reference 11). In addition, the purified serum IgG of HIV-1–ITP patients is 150-fold more reactive than serum, compared with the 3–5-fold greater reactivity reported for purified IgG blocking Ab in normal subjects (18). It is possible that anti–GPIIIa 49–66 was originally a polyreactive natural Ab that underwent somatic mutation and selective pressure by antigens (HIV-1), as has been suggested for the development of autoimmune disease (17). This is supported by the presence of lower affinity (Kd = 7–12 nM) Ab as well in our anti–GPIIIa 49–66 preparation (11). The same selective pressure could also apply for natural, polyreactive, low-affinity IgM "antiidiotype" Ab.

Alternatively, the pathogenic potential of low-affinity IgM Ab has recently been demonstrated in a study comparing monoclonal mouse IgM anti-RBC Ab with its IgG class-switch variant (20). In these studies, the RBC binding activity of the IgM Ab was 1,000 times that of its IgG class-switch variant and was related to its pentameric structure, which promoted binding, agglutination of RBCs, and hemolytic anemia. These data indicate that affinity maturation of autoantibodies may not be required for generation of autoantibodies capable of inducing clinical pathology. The same applies for the reactivity of the IgM antiidiotype of our study.

Although the role of antiidiotype Ab in the regulation of the immune response is controversial, a case can be made for its dysregulation in the pathophysiology of some autoimmune diseases: (a) patients with severe, uncontrolled SLE have high levels of anti-DNA Abs and low levels of anti-$F(ab')_2$ Abs, whereas patients with quiescent disease have the reverse (21); (b) patients with systemic vasculitis have antiidiotype Abs against antimyeloperoxidase and antineutrophil cytoplasmic antigen, with rise in antiidiotype titer as disease activity subsides (22, 23); and (c) a hemophiliac patient with a serious anti–factor VIII (antihemophilic factor) inhibitor developed antiidiotype Ab against anti–factor VIII, which coincided with recovery and the disappearance of the inhibitor (24). A case for antiidiotype dysregulation is indirectly supported by other observations:

(a) patients with thyroid autoimmunity have naturally occurring antiidiotype IgM Ab against antimicrosomal Abs (25); (b) patients with myasthenia gravis have naturally occurring antiidiotype Abs against acetylcholine receptor Ab (26); and (c) intravenous γ-globulin infusions containing "antiidiotype" Ab are often effective in the treatment of patients with autoimmune thyroid disease (25), systemic vasculitis (22, 23), myasthenia gravis (27), and kawasaki syndrome (28).

Our results contribute to and extend these observations in a more definitive manner in HIV-1–ITP. Our observations demonstrate the presence of high-affinity anti–GPIIIa 49–66 Ab (11, 12), specific blocking IgM antiidiotype Ab, a positive correlation between IgM antiidiotype and platelet count, and most importantly, a reversal of in vivo antibody-induced thrombocytopenia with its antiidiotype. These data support the concept that dysregulation of antiidiotype Ab can play a role in the development of autoimmune disease in HIV-1–ITP.

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