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Immunology

Sequestration of anti-platelet GPIIIa antibody in rheumatoid factor immune complexes of human immunodeficiency virus 1 thrombocytopenic patients

(AIDS/purpura/CD5$^+$ B cells)

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ABSTRACT Human immunodeficiency virus 1-related idiopathic thrombocytopenic purpura (HIV-1-ITP) patients have a 4-fold increased percentage of CD5$^+$ B cells and a 4.8-fold increased percentage of serum immune complexes precipitated by polyethylene glycol (PEG-ICs) compared to control subjects, as reported previously. Since CD5$^+$ B cells produce predominantly IgM rheumatoid factor (RF) vs. Fc of IgG and PEG-ICs contain high levels of IgM, we looked for the presence of RF in the immune complexes of HIV-1-ITP patients. PEG-ICs were adsorbed to protein A and dissociated with acid, and IgM and IgG were purified by gel filtration and affinity chromatography. Solid-phase ELISA was used to measure antibody specificity vs. platelets, Fc, and HIV-1 gp120, p24, and CD4. Dissociated IgG antibody reacted with platelets, HIV-1 gp120, p24, and CD4, but not with Fc. Serum IgG did not react with platelets or Fc but did react with HIV-1 gp120, p24, and CD4. Both PEG-IC IgM and serum IgM reacted with Fc as well as the other four antigens. Control IgM and IgG were unreactive. Isolated IgM from PEG-ICs relocated ≈50% of the IgG preincubated with IgM to the V_o region of a G200 gel-filtration column. Anti-platelet IgG but not IgM could be affinity-purified from fixed platelets. Both F(ab′)$_2$ fragments of anti-platelet IgG and the total PEG-IC bound to platelets in a saturation-dependent manner. F(ab′)$_2$ of anti-platelet IgG inhibited 50% binding of PEG-IC to platelets at an F(ab′)$_2$/complex ratio of 3:1 (wt/wt). Scatchard analysis revealed two classes of binding sites: high-affinity K_d values of 0.8–1.8 nM and lower-affinity K_d values of 6.6–12.3 nM with respective numbers of binding sites of 44,000–57,000 and 122,000–256,000 (n = 4). Anti-platelet IgG of 6/6 patients precipitated GPIIIa from platelet lysates of surface ^{125}I-labeled platelets. Platelet count correlated inversely with anti-platelet IgG ($r = -0.73$; $P < 0.01$; $n = 27$). Thus, PEG-ICs of HIV-1-ITP patients contain IgM RF, which sequesters serum anti-platelet IgG containing anti-GPIIIa. Anti-platelet IgG contributes to binding of immune complexes to platelets and correlates with thrombocytopenia.

Patients with human immunodeficiency virus 1 (HIV-1) infection develop an immunologic thrombocytopenia (idiopathic thrombocytopenic purpura; HIV-1-ITP) that is clinically indistinguishable from classic autoimmune thrombocytopenia (1) with respect to treatment, presence of normal to increased megakaryocytes in their bone marrow, deposition of immunoglobulin on their platelets (2), and kinetic evidence for both increased platelet destruction (3–5) and impaired platelet production (4, 5).

The disease is different with respect to the markedly elevated IgG, IgM, and C3C4 on their platelets (2, 6) and the presence of PEG-precipitable serum immune complexes containing IgG, IgM, and C3C4 (7). Our laboratory has provided evidence for the deposition of immune complexes from their sera onto their platelets (7, 8). The complexes contain anti-F(ab′)$_2$ (9), anti-gp120 (7), and anti-idiotype anti-HIV-1 gp120 antibodies (7, 10). The anti-HIV-1 gp120 anti-idiotype antibody correlates with thrombocytopenia.

We have recently noted an increased incidence of CD5$^+$ B cells (also designated, B-1) in HIV-1-infected patients, which was particularly elevated in thrombocytopenic patients and correlated with thrombocytopenia as well as PEG-precipitable serum immune complexes (11). CD5$^+$ B cells generally produce low-affinity antibodies, which are predominantly rheumatoid factors (RFs) of the IgM class against Fc of IgG (12–14). Many of the antibodies have several specificities and some are autoreactive (12–14). CD5$^+$ B cells are elevated in several autoimmune disorders as well as genetically autoimmune mice (13, 15–18). They have also been implicated in formation of a primordial idiotype–anti-idiotype network (12–14).

We therefore investigated the possibility that the high concentration of IgM-containing immune complexes found in the sera and on the platelets of HIV-1-ITP patients may be due at least in part to cross-reactive RFs and other low-affinity multispecific antibodies.

The present report documents the presence of IgM RF as well as the unexpected presence of anti-platelet GPIIIa in the immune complexes of HIV-1-ITP patients.§ RF reacts with serum IgG and sequesters anti-platelet IgG.

MATERIALS AND METHODS

Population. The population studied consisted of 27 HIV-1-seropositive subjects without AIDS. Fifteen were thrombocytopenic (7 homosexuals and 8 intravenous drug abusers) and 12 had normal platelet counts (6 homosexuals and 6 drug abusers). Five healthy control subjects were also studied.

Purified IgG. IgG was prepared from serum by ion-exchange chromatography from all 32 subjects studied (9).

F(ab′)$_2$. F(ab′)$_2$ fragments were prepared from purified IgG by pepsin digestion as described (9).

Fc. Fc fragments were prepared by papain digestion (20). The digest was affinity-purified on a staphylococcal protein A column equilibrated in 0.01 M phosphate-buffered saline (PBS), eluted with 0.1 M glycine buffer (pH 2.5), and verified by SDS/PAGE.

Immune Complexes. Immune complexes were prepared from serum by PEG precipitation (8). Precipitates were dissolved in 1/5 their serum volume in PBS.

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Abbreviations: HIV-1-ITP, human immunodeficiency virus 1-related idiopathic thrombocytopenic purpura; PEG-IC, polyethylene glycol-precipitable immune complex; RF, rheumatoid factor; r, recombinant.
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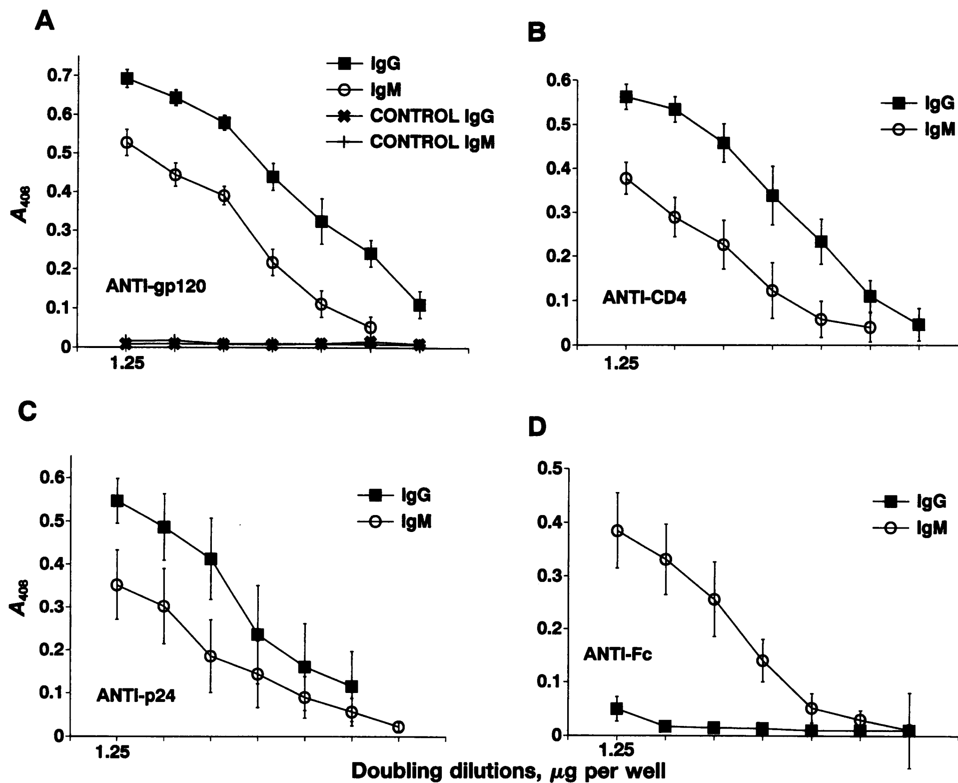

FIG. 1. Binding of dissociated IgG and IgM of PEG-ICs to gp120 (*A*), rCD4 (*B*), rp24 (*C*), and Fc fragment of human IgG (*D*). The respective antigens were applied to plastic microtiter plate wells at 1.25 μg per well and the IgG or IgM was serially diluted with doubling dilutions in PBS. Unbound immunoglobulin was washed away in PBS/1% BSA and bound IgG and IgM were measured by ELISA with alkaline phosphatase-conjugated goat anti-human IgG and IgM, respectively ($n = 4$ different patients; SEM is shown).

Isolation of Ig and IgM from Immune Complexes. PEG-precipitable immune complexes (PEG-ICs) were applied to a staphylococcal protein A affinity column. 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 G200 gel-filtration column preequilibrated with the same elution buffer. Effluents of the IgM and IgG peaks were isolated, neutralized, dialyzed against PBS, and then applied to rabbit anti-IgG and anti-IgM affinity columns, respectively, prepared with Affi-Gel 10 (Bio-Rad). The flow-through material was free of contaminating IgG or IgM as determined by immunoblot as well as ELISA. The ratio of IgM to IgG in the complex was 1:4, as determined by protein concentration utilizing the Bio-Rad reagent with standard IgM and IgG proteins.

ELISA. Antibody reactivity was measured by solid-phase ELISA with serial dilutions of IgG or IgM on U-shaped polyvinyl microtiter plates (Curtin Matheson Scientific, Wayne, NJ) preincubated overnight at 4°C with 200 ng of recombinant gp120 (Repligen), p24 (Bioservices, National Institute of Allergy and Infectious Diseases, Rockville, MD), CD4 (courtesy of S. Chamov, Genentech), Fc in 0.1 M bicarbonate buffer (pH 9.5), or 1×10^7 washed platelets in PBS and blocked with 1% bovine serum albumin (BSA) in PBS. For IgG reactivity, the second antibody was a 1:500 dilution of goat $F(ab')_2$ anti-human IgG (γ-chain specific) coupled to alkaline phosphatase (Sigma); for IgM reactivity, the second antibody was a 1:500 dilution of goat anti-human IgM (μ-chain specific) similarly coupled (Sigma). Color was read in an automated microtiter plate reader at 408 nm. Antibody potency was compared by extrapolating the doubling dilution curve to baseline on the immunoglobulin concentration axis. Thus, the lower the concentration in nanograms per well, the more potent the antibody.

Affinity Purification of Anti-Platelet IgG. Washed platelets (1×10^8) (21) were fixed with 2% paraformaldehyde overnight at 4°C, washed extensively with Ringer's 2 mM EDTA, and sedimented to remove supernatant. Purified immunoglobulin (0.4 mg) from PEG-ICs, in 1 ml of PBS, was added to the platelet pellet at room temperature for 2 hr followed by overnight gentle rocking at 4°C. The platelets were sedimented, washed three times with Ringer's 2 mM EDTA (21), and then eluted with 0.1 M glycine buffer (pH 2.5). The eluate was neutralized with 1 M Tris buffer and dialyzed against PBS.

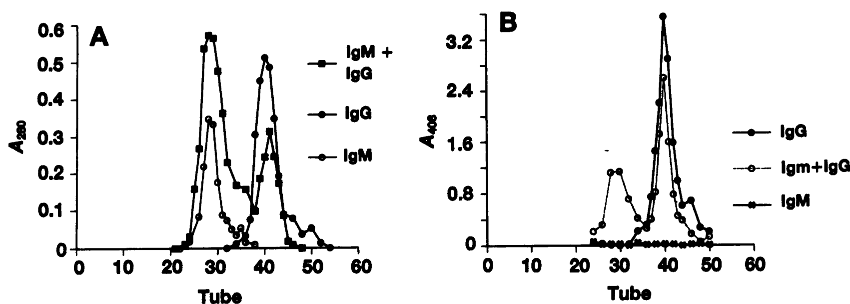

FIG. 2. Sequestration of immune complex-dissociated IgG by immune complex-dissociated IgM on gel filtration. IgG + PBS, IgM + PBS, or IgM and IgG (1:4, wt/wt) was incubated for 2 hr at room temperature and then applied to a G200 gel-filtration Sephadex column equilibrated in PBS. (*A*) Elution of dissociated IgM, IgG, and reassociated IgM + IgG on a G200 gel-filtration column. Effluents were monitored at A_{280}. (*B*) IgG elution of dissociated IgM, IgG, and reassociated IgM + IgG on a G200 gel-filtration column. Effluents were monitored by IgG ELISA reactivity ($n = 4$).

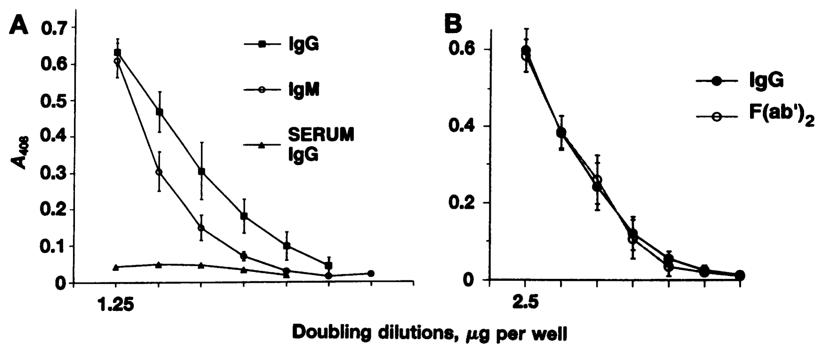

FIG. 3. Anti-platelet reactivity of dissociated IgG and IgM of immune complexes compared to HIV-1-ITP patient serum IgG. Washed platelets (1×10^7) were applied to plastic microtiter wells in PBS for 1 hr at room temperature, blocked with PBS/1% BSA, and then assayed by ELISA for IgG or IgM as in Fig. 1. (*A*) Anti-platelet reactivity of dissociated IgG and serum IgM. (*B*) Anti-platelet reactivity of dissociated F(ab')$_2$ fragments of IgG vs. serum IgG (n = 4).

Binding of Immune Complexes to Platelets on Microtiter Plates. Platelets (1×10^7) were made adherent to microtiter plates as described above, blocked with 3% non-fat milk in PBS, and then incubated with doubling dilutions of PEG-IC (solubilized in PBS), starting at 7 μg per well for 1 hr at room temperature, in the absence and presence of 7 μg of F(ab')$_2$ of affinity-purified anti-platelet IgG. Platelets were then washed with 3% Blotto/PBS and reacted with ≈100,000 cpm of ^{125}I-labeled protein A (30 mCi/mg; 1 Ci = 37 GBq; Amersham-Searle) for 1 hr at room temperature. Unbound ^{125}I-protein was washed away in 3% Blotto/PBS; the wells were then removed with a heated wire cutter and assayed for radioactivity.

Binding of Affinity-Purified Anti-Platelet IgG to Platelets in Suspension. Affinity-purified anti-platelet IgG (40 μg) was radiolabeled with ^{125}I by the Iodo-Gen (Sigma) method (22). One hundred microliters of ^{125}I-anti-platelet IgG (2.1 μCi/μg in PBS/2% BSA) was added to 100 μl of 1×10^7 washed platelets in PBS and incubated overnight at 4°C at various dilutions in PBS from 0.05 to 3 μg/200 μl. The total 200 μl plus an additional 200 μl of PBS wash was then carefully applied to the top of a polypropylene test tube (12 × 75 mm) (Sarstedt) containing 4 ml of a 20% sucrose cushion in PBS. The mixture was centrifuged at 12,000 × g for 15 min at 4°C. The supernatant was removed by aspiration, the inside of the test tube was carefully wiped with a cotton swab, and the platelets in the test tube were monitored for radioactivity. Nonspecific binding was assessed by adding 30-fold nonradioactive anti-platelet IgG to block specific binding.

Immunoprecipitation of Surface ^{125}I-Labeled Platelet Membrane with Affinity-Purified Anti-Platelet IgG. Gel-filtered platelets were labeled with ^{125}I by the lactoperoxidase method (23) and lysed in 1% Triton X-100/0.01 M Tris·HCl buffer, pH 8.0, containing 1 mM iodoacetamide, 2 mM phenylmethylsulfonyl fluoride, 0.14 M NaCl, 10 mM EDTA, 10 mM benzamidine, and 5 μM soybean trypsin inhibitor at a final concentration of 3×10^8 platelets per ml. The lysate was kept at 4°C for 1 hr and centrifuged in a microcentrifuge for 5 min at 4°C. SDS was added to the supernatant at a final concentration of 0.1% and the supernatant was cleared by incubating with protein A-Sepharose [100-μl packed volume in 0.01 M Tris buffer (pH 8.0)] for 1 hr at 4°C. One hundred microliters of supernatant was treated with 100 μg of affinity-purified anti-platelet antibody or 20 μg of monoclonal antibodies LK3r or LK7r against GPIIIa in 100 μl for 2 hr at 4°C on a rocker. Washed protein A-Sepharose [in 0.01 M Tris·HCl (pH 8.0) containing 0.14 M NaCl and 0.02% NaN_3] was added overnight at 4°C on a rocker. The suspension was centrifuged to obtain the pellet, which was washed twice with 0.1% Triton X-100 in the same buffer solution, once in the buffer solution alone, and once in 0.5 M Tris·HCl (pH 6.8). Fifty microliters of sample buffer [2.4% SDS in 0.5 M Tris·HCl (pH 6.8) containing 12% (vol/vol) glycerol] was added to the pellet in a microcentrifuge tube, heated at 100°C for 5 min, and centrifuged to obtain the supernatant. An aliquot was reduced with 5% 2-mercaptoethanol by incubating at 37°C for 1 hr. The supernatants were applied to SDS/10% polyacrylamide gels; the gels were dried

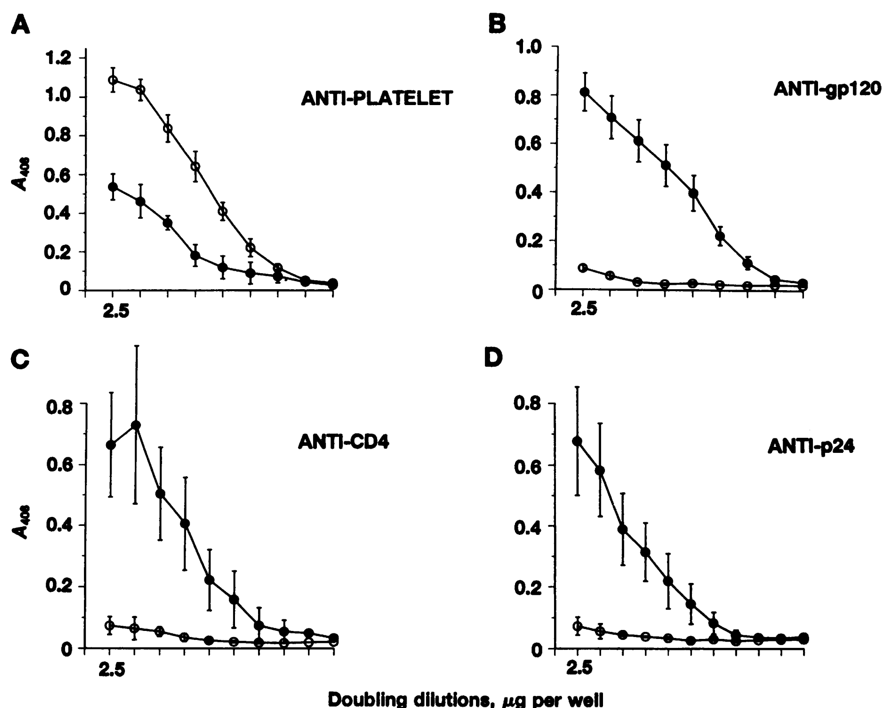

FIG. 4. ELISA reactivity of immune complex-dissociated IgG (●) vs. affinity-purified anti-platelet IgG (○) of the dissociated complex with platelets (*A*), rgp120 (*B*), rCD4 (*C*), and rp24 (*D*). Platelets (1×10^7) were adsorbed to plastic microtiter wells as in Fig. 3. rgp120, rCD4, and rp24 were applied to plastic microtiter wells as in Fig. 1 (n = 4).

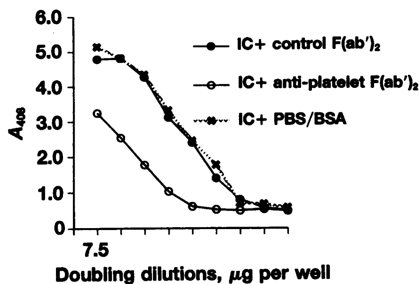

FIG. 5. Inhibition of binding of immune complexes to platelets with $F(ab')_2$ fragments of affinity-purified anti-platelet IgG of the dissociated complex. PEG-ICs were preincubated with PBS/1% BSA, control $F(ab')_2$, or affinity-purified anti-platelet $F(ab')_2$ for 2 hr at room temperature before incubation with platelets adsorbed to plastic microtiter wells as in Fig. 4. Reactivity was measured by addition of ^{125}I-staphylococcal protein A [which did not bind to $F(ab')_2$ fragments] ($n = 4$).

under vacuum and exposed to Kodak X-Omat imaging film for autoradiography.

RESULTS

Fig. 1 demonstrates IgG and IgM antibody reactivity obtained from the dissociated immune complexes of HIV-1-ITP patients against recombinant (r) gp120, CD4, and p24 and against Fc of IgG. IgG did not react with Fc, whereas IgM did, indicating the presence of IgM RF in the complex. Similar results were noted with purified serum IgG and IgM (data not shown). Control IgG and IgM did not bind to any of the antigens tested.

Fig. 2 demonstrates the ability of dissociated IgM to bind to and relocate dissociated IgG on a G200 gel-filtration column. Note that ≈50% of the IgG migrates with the IgM peak after preincubation of IgM and IgG.

Fig. 3*A* demonstrates the ability of dissociated IgG as well as IgM to bind to platelets. Fig. 3*B* demonstrates reactivity of $F(ab')_2$ fragments of IgG for platelets, indicating the absence of nonspecific binding via Fc receptors on platelets. No anti-platelet reactivity was detectable in serum-purified IgG of HIV-1-ITP patients, despite its presence in their PEG-ICs (data not shown; $n = 15$).

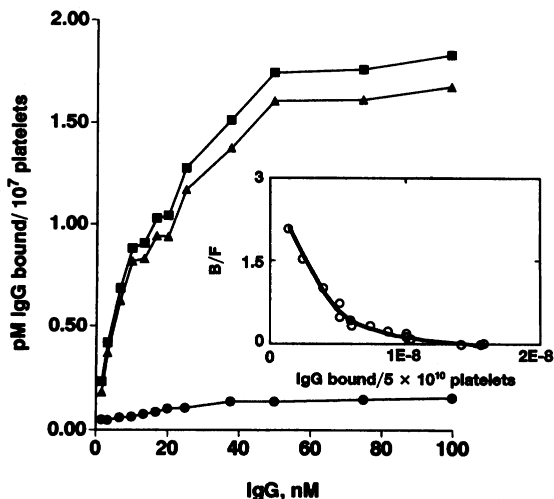

FIG. 6. Saturation binding and Scatchard analysis of affinity-purified ^{125}I-anti-platelet IgG to washed platelets. Total binding (■), nonspecific binding (●), and specific binding (▲) are plotted. (*Inset*) Scatchard plot of bound/free (B/F) ratio vs. IgG bound/5 × 10^{10} platelets. K_d and binding sites were calculated by using the LIGAND computer program. 1E-8, 1 × 10^{-8} M, etc.

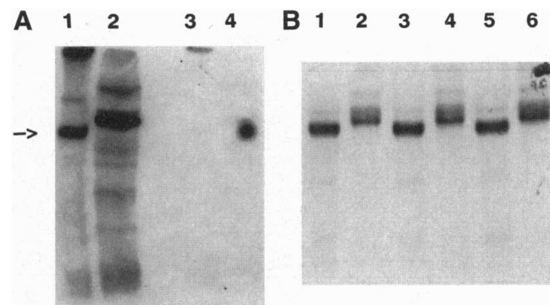

FIG. 7. Immunoprecipitation of ^{125}I-platelet surface antigens with anti-platelet IgG. (*A*) Experiment 1. Lanes 1 and 2 contain platelet lysates of RM (homosexual ITP) treated with anti-platelet IgG, unreduced and reduced, respectively; lanes 3 and 4 were treated with control IgG, unreduced and reduced, respectively. (*B*) Experiment 2. Lanes 1, 3, and 5 are unreduced. Lanes 2, 4, and 6 are reduced. Lanes 1 and 2, anti-platelet IgG of MM (intravenous drug abuser ITP); lanes 3 and 4, treated with monoclonal antibody LK3r against GPIIIa; lanes 5 and 6, treated with monoclonal antibody LK7r against GPIIIa. Control IgG (not shown) gave no precipitation as in experiment 1, lanes 3 and 4. Arrow, 100-kDa band.

Fig. 4*A* demonstrates affinity purification of anti-platelet IgG from fixed platelets. Anti-platelet IgM could not be affinity-purified. Eluted IgM remained polyreactive, reacting with platelet, gp120, CD4, and p24 (data not shown). The affinity-purified anti-platelet IgG did not react with gp120, CD4, and p24 (Fig. 4 *B–D*). Lack of reactivity was also noted with HIV-1-impregnated beads in a standard ELISA (data not shown).

Fig. 5 demonstrates that complexes containing anti-platelet IgG can bind to intact platelets and that this can be inhibited by $F(ab')_2$ fragments of anti-platelet IgG. Inhibition of 50% binding of immune complex to platelets was obtained at an $F(ab')_2$/complex ratio of 3:1.

Fig. 6 depicts the saturation binding of affinity-purified ^{125}I-anti-platelet IgG to platelets. Scatchard analysis revealed two classes of receptors with K_d values of 1.8 and 6.6 nM and number of receptor sites at 57,000 and 129,000. Similar studies on three additional patients revealed high-affinity K_d values of 0.84–1.8 nM, low-affinity K_d values of 6.6–12.3 nM, number of high-affinity receptor sites at 44,000–57,000, and number of low-affinity receptor sites at 122,000–256,000.

Since Scatchard analysis revealed the presence of 44,000–57,000 high-affinity receptors per platelet, we elected to search for the epitope(s) by immunoprecipitation of lysates from surface ^{125}I-labeled intact platelets. Fig. 7 demonstrates immunoprecipitation of a major band with properties of platelet GPIIIa—i.e., 100 kDa on SDS/PAGE, unreduced; 117 kDa, reduced—with identical locations of GPIIIa immunoprecipitated with two IgG1-K murine monoclonal antibodies raised against GPIIIa, LK3r, and LK7r. Other minor bands were occasionally noted, particularly in experiment 1, where the autoradiographic bands were intense. Similar results were obtained in four additional patients (data not shown).

Fifteen thrombocytopenic [58,000 ± 8000 (mean ± SEM) platelets per μl] and 12 nonthrombocytopenic (218,000 ± 18,000) patients were compared. HIV-1-ITP patients had 5.4-fold greater PEG-ICs than 7 control subjects (0.91 ± 0.4 vs. 0.19 ± 0.02 mg/ml), with intermediate values for nonthrombocytopenic patients. Compared to nonthrombocytopenic patients, HIV-1-ITP patients had 3.8-fold greater dissociated IC IgM (219 ± 22 ng of IgM capable of detecting antigen vs. 58 ± 8 ng) and 2.4-fold greater dissociated IC anti-platelet IgG (189 ± 19 ng of IgG vs. 78 ± 13 ng), respectively ($P < 0.0001$ for all differences).

Correlations were examined for permutations of all four parameters. Linear regression correlations were considered relevant if statistically significant, if concordance was observed between HIV-1-ITP and the total HIV-1 group, and if the r value was >0.5. This was noted for platelet count vs. IgG anti-platelet antibody ($r = 0.73$; $P < 0.01$; $n = 15$ for HIV-1-ITP; $r = 0.72$; $P < 0.001$; $n = 27$ for the total HIV-1 group).

DISCUSSION

These data indicate the presence of IgM RF and anti-platelet IgG in PEG-ICs of HIV-1-infected patients. The levels in thrombocytopenic patients were greater than in nonthrombocytopenic patients. Although RF was present in sera as well as immune complexes, anti-platelet IgG was sequestered in the immune complexes, presumably by RF, and was not detectable in patient sera.

RF was shown to be present in HIV-1 and HIV-1-ITP patients by the ability of their affinity-purified serum and PEG-IC-dissociated IgM to bind to Fc. RF was found to bind IgG as demonstrated by its ability to relocate IgG to the high molecular weight region (V_o) of a G-200 gel-filtration column. The presence of RF in sera and PEG-ICs may have been predicted from our recent observation on the increased percent and absolute number of $CD5^+$ B cells in HIV-1-infected patients, particularly when thrombocytopenic, since $CD5^+$ B cells produce IgM RF (12–15). HIV-1-ITP patients have a 4-fold increased percentage of $CD5^+$ B cells compared to control subjects (11). A similar increase in percentage of $CD5^+$ B cells and serum RF has been reported for patients with autoimmune disease—rheumatoid arthritis (13, 15, 16), Sjogren syndrome (24), and primary antiphospholipid syndrome (25)—as well as in genetically autoimmune mice (17, 18). The ability of individual IgG as well as IgM antibodies produced by $CD5^+$ B cells to have multiple specificities could account for the primordial idiotype–anti-idiotype complexes associated with these cells (12–14). The same may apply to the anti-idiotype anti-HIV-1 gp120 antibodies described in HIV-1-ITP patients (7, 10) and the abundance of serum immune complexes in this disorder (2, 6–9, 26). It should be recognized that RF may also be produced by $CD5^-$ B cells. However, these are likely to be of the B-1b variety, which have been shown to contain the mRNA of $CD5^+$ B cells (25).

This work documents monomeric anti-platelet IgG in an autoimmune platelet disorder, as demonstrated by DEAE purification, 7S location on gel filtration, $F(ab')_2$ binding, affinity purification, saturation binding, and Scatchard analysis. 7S anti-platelet IgG was detectable in the PEG-ICs of patients but not in their sera. The absence of anti-platelet IgG in the sera of HIV-1-ITP homosexual patients has been reported by our group (2). The finding of anti-platelet IgG in their PEG-ICs was therefore unexpected. Difficult to explain was anti-platelet IgG in the sera of 6 of 13 narcotic addict HIV-1-ITP patients (6) and in 8 of 8 hemophiliac HIV-1-ITP patients (24) [proven by $F(ab')_2$ binding]. A possible explanation could be the level of RF in the three HIV-1-ITP cohorts, with perhaps higher levels of RF in the homosexual cohort studied. This appears to be true for 8 hemophiliac HIV-1-ITP patients who have 2.8-fold less RF than the 15 patients of this study. The inability to detect serum anti-platelet IgG in the homosexual cohort could then be due to competition between serum RF and high-affinity anti-platelet IgG for platelets.

Affinity-purified anti-platelet IgG did not cross-react with the HIV-1 antigens rgp120, rCD4, and rp24 or with HIV-1-impregnated beads, making it unlikely that the anti-platelet IgG represents molecular mimicry of antibody for HIV-1 antigens as recently reported for one patient with HIV-1-ITP (27). Affinity-purified anti-HIV-1 gp120 did not bind to platelets (7, 8). However, anti-HIV-1 gp120 antibody is present in the eluates and PEG-ICs of HIV-1-ITP patients and is therefore likely to be present as a complex on their platelets (7, 8, 10). Affinity-purified anti-platelet IgG did react with platelet GPIIIa in 6 of 6 patients. GPIIIa was the major reactive band on immunoprecipitation of the surface platelet membrane. This is supported by the number of high-affinity receptors found on Scatchard analysis, which is similar to the 47,000–57,000 sites reported for GPIIb/IIIa (28).

In a previous study (29), we reported that HIV-1-ITP PEG-IC binding to platelets was not due to Fc binding (i.e., not blocked by monoclonal antibody IV.3 vs. platelet $FcR_{\gamma}II$ or by aggregated IgG) but was due in part to complement receptor binding (i.e., inhibition of binding by Fab anti-Clq, anti-C3, or anti-C4 antibody of ≈50%). The present data indicate that these ICs bind to platelets via $F(ab')_2$ anti-platelet IgG as well. This does not rule out the additional possibility that serum RF could bind to platelet-bound anti-platelet IgG.

Anti-platelet IgG correlated strongly with thrombocytopenia. These data are compatible with anti-platelet IgG (anti-GPIIIa) playing a role in the induction of thrombocytopenia in HIV-1-ITP patients by binding to platelet GPIIIa directly as well as contributing to the binding of immune complexes to platelets.

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