Presence of Cross-Reactive Antibody Between Human Immunodeficiency Virus (HIV) and Platelet Glycoproteins in HIV-Related Immune Thrombocytopenic Purpura

By Ali Bettaieb, Patricia Fromont, Fawzia Louache, Eric Oksenhendler, William Vainchenker, Najib Duédari, and Philippe Bierling

We previously reported the presence in platelet eluates of autoantibodies directed against epitopes of the platelet glycoprotein (GP)Ib/IIa complex in acquired immunodeficiency syndrome (AIDS)-free human immunodeficiency virus (HIV)-infected patients with immunologic thrombocytopenic purpura (ITP). We investigated whether HIV antibodies recognized platelet membrane antigens to determine whether the virus might be directly or indirectly responsible for the thrombocytopenia in this context. Direct eluates of platelets from 25 patients with HIV-related ITP contained IgG reacting with HIV-GP160/120 and also, in 45% of patients, detectable antiplatelet antibodies, immunochemically characterized as anti-GPIIb and/or anti-GPIIa in 5 patients. Furthermore, serum HIV-GP160/120 antibodies could be absorbed on and eluted from platelets from normal non–HIV-infected healthy blood donors (indirect eluates). In contrast, GP160/120 antibodies present in the serum of nonthrombocytopenic HIV-infected patients were not absorbable on normal platelets in most patients, suggesting a pathogenic role in HIV-related ITP. We performed detailed studies of a patient with the highest titer of both HIV-GP160/120 and GPIIb/IIa antibodies in direct and indirect platelet eluates. No antibody binding to GPIIb/IIa-deficient Glanzmann thrombasthenic platelets was detected. Furthermore, binding/elution experiments conducted with insoluble recombinant GP160 (expressed in baculovirus) and purified platelet GPIIb/IIa demonstrated that the patient’s IgG bound specifically, through the F(ab')2 portion, to a common epitope of HIV-GP160/120 and platelet GPIIb/IIa. This common epitope was present on a recombinant GP160 expressed in baculovirus but absent from another recombinant GP160 expressed in vaccinia virus, suggesting that the cross-reactivity is dependent on the glycosylation or conformational structure of the GP. We conclude that molecular mimicry between HIV-GP160/120 and platelet GPIIb/IIa may explain at least some cases of ITP in AIDS-free HIV-infected patients.

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IDIOPATHIC thrombocytopenic purpura (ITP) is a frequent hematologic disorder characterized by immunologic platelet destruction. ITP most often results from the presence of platelet autoantibodies directed mainly against epitopes localized on the glycoprotein (GP)Ib/IIa complex.1–5 A syndrome similar to ITP frequently occurs in HIV-infected patients regardless of the risk group to which they belong, eg, homosexual men,6,9 intravenous drug abusers,9,10 and hemophiliacs.11,12 Immunologically mediated platelet destruction has been clearly demonstrated in such patients,13 but the precise mechanism of the thrombocytopenia has not yet been established. Two main hypotheses have been advanced. The first hypothesis implicates nonspecific deposition of circulating immune complexes on the platelet surface with subsequent clearance of the opsonized platelets.7 This hypothesis is supported by the presence of anti-HIV antibodies and the absence of detectable HIV antigens on the platelet surface.14 Certain evidence indicates that anti-F(ab')2 antibodies directed against HIV antibodies (antidiotypic antibodies) might play a role in the formation of such immune complexes.15 The second hypothesis, supported by the findings of two independent teams of investigators, is based on the presence of specific platelet antibodies. Thus, von dem Borne et al16,17 reported the presence of platelet autoantibodies in most HIV-infected patients with or without thrombocytopenia and concluded that the nature of these autoantibodies was not different from that of the autoantibodies observed in classic ITP. We demonstrated that ITP in HIV-infected AIDS-free patients results, at least in some patients, from the presence of platelet autoantibodies directed mainly against epitopes localized on platelet GPIIb/IIa.18

To reconcile the two hypotheses, we investigated the possible involvement of molecular mimicry between HIV and platelet antigens. Our results, showing cross-reactivity of HIV antibodies with platelet GPIIb/IIa, could explain some cases of ITP in HIV-infected patients.

PATIENTS AND METHODS

Patients. Blood samples were obtained from HIV-infected patients with (n = 28) or without (n = 11) ITP. The presence of HIV antibodies was demonstrated by enzyme-linked immunosorbent assay (ELISA) and Western blotting analysis in the serum of all subjects. The patients with ITP belonged to the following risk groups: drug addicts (n = 18), homosexual men (n = 7), and transfusion recipients (n = 3). We previously reported immunohematologic findings in 22 of these 28 patients, including the three whose platelet eluates precipitated GPIIb/IIa.18 Among the nonthrombocytopenic patients, five were homosexual men, four were drug addicts, and two were female sexual partners of seropositive men. The patients with ITP satisfied the following diagnostic criteria: isolated thrombocytopenia with a platelet count less than 50 x 10^9/L, an increased number of megakaryocytes in an otherwise normal bone marrow (BM) aspirate, absence of Centers for Disease Control (CDC)-defined AIDS and absence of other causes of peripheral thrombocytopenia (eg, thrombotic thrombocy-
topenic purpura, disseminated intravascular coagulation, hyper-splenism). The clinical characteristics of most of the patients with HIV-related ITP have been described elsewhere.9,19,20

Patient Bou, born in 1960, was a drug addict from 1978 to 1983. HIV seropositivity (CDC stage IVC2) and ITP were diagnosed in 1988. Treatment with zidovudine was started in August 1988 and the platelet count increased to near-normal levels (142 \times 10^9/L). Pulmonary tuberculosis, diagnosed in January 1990, resolved with specific treatment.

Platelet autoantibody determination. Platelet-associated IgG (PAIgG) was detected according to the method described by von dem Borne et al.21 Samples (sera, eluates or F(ab')2 fragments) were tested for antiplatelet membrane activity by means of an indirect immunofluorescence test (PIFT) on paraformaldehyde-de-
treated platelets22 with fluorescein-conjugated F(ab')2) antigen (\gamma) IgG or anti-thrombin light chains (Dakopatts, Copenhagen, Denmark).

In some cases, sera and eluates were also studied in immunopre-
precipitation experiments. Platelets 1 \times 10^9 were surface-labeled with ^{125}Iodine using the lactoperoxidase catalyzed method.23 The platelets were subsequently solubilized in 1 mL buffer A [containing Tris-HCl 20 mmol/L NaCl 150 mmol/L (Tris-buffered saline-TBS), Triton X-100 1%, EDTA 5 mmol/L, phenylmethylsulfonyl fluoride (PMSF) 2 mmol/L, and 0.5 \mu g/mL leupeptin] for 30 minutes at 4°C, and the lysate was centrifuged for 30 minutes at 17,600g at 4°C. Fifty microliters of lysate were then incubated overnight at 4°C with 50 \mu L serum, 100 \mu L eluate, or 10 \mu L purified monoclonal antibody (MoAb). Two hundred microliters of a 10% suspension of protein A-Sepharose (Pharmacia, Paris, France) were added to the lysate and incubated for 1 hour at 4°C. The beads were then washed five times with buffer A, two times with buffer B supplemented with 0.2 mol/L NaCl, and two times with buffer C supplemented with 0.35 mol/L NaCl. Fifty microliters of 2% sodium dodecyl sulfate (SDS) and 5% \beta-mercaptoethanol were added to each pellet and, after boiling for 5 minutes, the solutions were analyzed on a 7.5% sodium dodecyl sulfate (SDS)-polyacryl-
amide gel according to the method of Laemmli.24 Gels were then fixed, dried, and autoradiographed.

Platelet eluates. Either elution of PAIgG was performed accord-
ing to the method of Helmerhorst et al.25 The direct eluates were then tested for antiplatelet and anti-HIV activity in different techniques in comparison with the last platelet washing fluid to detect possible contamination of the eluates by serum. Results were interpreted only if the last platelet washing sample was negative. Negative controls included direct eluates of platelets from normal individuals and HIV-negative patients with ITP.

HIV antibody determination. HIV antibodies were detected using a commercially available enzyme-linked immunosorben-
assay (ELISA) kit (Abbott Laboratories, Paris, France) and by Western blot immunoassay (Du Pont De Nemours, Les Ulis, or Diagnostics Pasteur, Marne-La-Coquette, France). The ELISA kit contained mainly GP41 and GP24 recombinant-HIV proteins, with small amounts of GP160/120 and GP55 of HIV1 and GP41 of HIV2. The ELISA was considered positive if the ratio of sample absorbance to positive control absorbance was greater than or equal to the calculated optical density (OD) cutoff value (the cutoff value was calculated according to the manufacturer’s recommenda-
tions using the negative and positive controls included in the commercial kit). In the HIV1 Western blot assay, eluates (diluted 1:40) were incubated with the test strips for 2 hours at room temperature and the bands were disclosed by an alkaline phosphatase-conjugated goat anti-human serum (Diagnostics Pasteur) or by a series of reactions with goat anti-human IgG conjugated with biotin, avidin conjugated with horseradish peroxidase (HRP), and the HRP substrate 4-chloro-1-naphthol (Dupont). The result was considered positive in these experiments when at least one band was visualized.

HIV-infected cell lines. HIV antibodies from patient Bou plate-
let direct eluates were detected by indirect immunofluorescence on methanol-permeabilized (1 minute at 22°C) cytocentrifuged U937 and CEM cell lines infected by the HTLV-IIB and Bru-HIV isolates, respectively.

Detection of HIV antigens on patient platelets. HIV antigens were sought on the patient’s platelets by immunoblotting,26 immu-
noprecipitation,18 and the indirect immunofluorescence procedure,22 using the following HIV MoAbs: anti-p24, anti-p17, and anti-Gp120 (provided by Dr R.M. Vazeux, Institut Pasteur, Paris, France). Two additional antibodies were used in the immunofluo-
rescence technique, one directed against p25, p34, and p65, (Genzyme, Boston, MA) and the second recognizing GP160/120 (Biosoft, Paris, France).

Immunoglobulin and F(ab')2 preparation. IgG was purified from patient Bou serum by means of the staphylococcal protein A-sepharose CL4B technique (Pharmacia, Paris, France), as described by Ely et al.27 Five milligrams IgG was then digested by 0.1 mg pepsin at pH 4.5 for 18 hours at 37°C. The F(ab')2 fragments were subsequently dialyzed against phosphate-buffered saline (PBS) and purified on an insoluble Protein A-column. Samples were subjected to SDS-polyacrylamide gel electrophoresis (SDS-
PAGE) to detect the possible presence of intact IgG in the F(ab')2 preparations. The F(ab')2 fragments were used in binding and elution experiments only if intact IgG was undetectable.

Platelet binding and elution experiments performed with patient sera. Washed platelets (10^9) harvested from healthy blood group O donors or GT patients were suspended in 500 \mu L PBS containing 3 mmol/L EDTA and 0.25% bovine serum albumin (BSA) and then incubated with 500 \mu L patient serum or plasma for 2 hours at room temperature. The mixture was subsequently washed in 20 mL of the same buffer four times with repeated centrifugation. The platelets were resuspended in 500 \mu L buffer, and 1 mL diethyl ether was added. The samples were then shaken for 2 minutes, incubated for 30 minutes at 37°C, and spun for 10 minutes at 3,000 g. The indirect eluates (aqueous solution) were tested in parallel with the last washing fluid for antiplatelet and anti-HIV activity. Results were interpreted only if the last platelet washing sample was negative.

Binding and elution experiments performed with eluates, sera, and F(ab')2 fragments on recombinant GP160 and purified platelets GPIb/IIa. Two recombinant GP160s (rGP160) of the HIV1 isolate (rGP160-1, rGP160-2), supplied by Drs M. Girard and M. Kaczorow (Pasteur Merieux, Val de Reuil, France) and by Repli-
gen (Cambridge, MA), respectively were studied. rGP160-1 is secreted into the culture medium of baby hamster kidney (BHK 21) cells infected by the recombinant vaccinia virus VV 1163.28 Amino acid sequencing has shown that rGP160-1 lacks 22 amino acids at position 696 to 717.28 rGP160-2 (derived from the IIb isolate of HIV1) is expressed as a full-length protein in insect cells by using a baculovirus expression vector and is neither processed nor secreted.29,30 Four hundred micrograms of the rGP160 was coupled to 0.5 mL Affi-gel 10 according to the manufacturer’s directives (BioRad, Paris, France).

Platelet GPIb/IIa was purified from human platelets as de-
scribed by Fitzgerald et al.11 with modifications. The platelet lysate was loaded onto a Concanavalin-A-ultrogel column. Filter materi-
als were eluted by 0.2 mol/L methyl-a-glucopyranoside (Serva, St.
Germain en Laye, France), and then dialyzed against TBS containing 0.1% Triton-X 100. Further purification was performed with insoluble anti-GPIIb MoAb (SZ21) (Immunotech, Marseille, France) coupled to a column of Affi-gel 10 (1 mg antibody/1 mL Affi-gel 10). GPIIb/IIIa complex was eluted from the column with 0.1 mol/L glycine, 0.1 mol/L NaCl, and 0.1% Triton X-100, pH 2.8, and the eluted fraction was immediately neutralized with 1 mol/L Tris. The purified GPIIb/IIIa was greater than 90% homogeneous as analyzed by SDS-PAGE. The only faint contaminant observed was the fibrinogen that was probably copurified with the GPIIb/IIIa. In addition, in an ELISA, the GPIIb/IIIa preparation failed to react with HLA class I, CD42b, CDw49b, and CD62 MoAbs coupled to 0.5 mL Affi-gel 10 according to the manufacturer’s directives (BioRad, Paris, France).

Absorption experiments with eluates, sera or F(ab')2 fragments were performed using Affi-gel 10 coupled to rGP160 or to purified GPIIb/IIIa. After several washes, absorbed materials were eluted by addition of an aqueous buffer containing 150 mmol/L H3PO4/100 mmol/L NaCl/1.5% BSA, pH 2.8, for 10 minutes at 4°C. The acidified suspension was centrifuged and then neutralized with 1 mol/L Tris. Eluted materials were tested for antiplatelet and anti-HIV activity in comparison with the last GP160 or GPIIb/IIIa Affi-gel column washing samples. Results were interpreted only if the last platelet washing sample was negative.

RESULTS

Platelet autoantibody determination. In accordance with a previous report,18 PAIgG levels were increased in 20 (80%) of the 25 patients with HIV-related ITP studied. For 9 of these 20 patients (45%), the direct eluate contained platelet antibodies that recognized in the PIIFT all the platelet panel except platelets from ABO compatible type I GT patients. Five of these positive eluates, including the three reported in our previous work,18 precipitated platelet GPIIb/IIIa (partially illustrated in Fig 3). In contrast, a weak increase in PAIgG was observed in only 2 of the 11 nonthrombocytopenic patients (18%) and was never elutable. Likewise, sera of 25 of the 28 thrombocytopenic but only 3 of the 11 nonthrombocytopenic patients were positive in the PIIFT.

Presence of HIV antibodies on platelets from HIV-infected thrombocytopenic patients. Direct platelet eluates from HIV-infected thrombocytopenic (n = 25) and nonthrombocytopenic patients (n = 11) were tested using ELISA and Western blot procedures for the presence of HIV antibodies (Fig 1). In the ELISA, 21 of the 25 eluates (84%) from thrombocytopenic patients were positive, whereas only 3 of the 11 eluates (27%) from nonthrombocytopenic patients were weakly positive. Furthermore, all 25 direct eluates from HIV-infected ITP patients were positive in the HIV Western blot immunoassay, whereas only four of the nonthrombocytopenic patients were positive (including two who were very weakly positive), and seven were negative. As shown in Fig 1, all 29 eluates positive in HIV-Western blot recognized HIV GP160/120, and 12 also recognized other proteins encoded by the Gag or Pol genes (p17, p24, p31, p53, p68).

Viral antigens on platelets of HIV-infected ITP patients. Immunoblotting and immunoprecipitation procedures with anti-HIV MoAbs were used to detect the presence of HIV antigens on HIV-infected ITP patient platelet extracts. Negative results were obtained for all four patients studied. Similar negative results were obtained with three other platelet samples in the indirect immunofluorescence test. These results do not strictly eliminate the presence of HIV antigens on the platelet membrane in these patients,

Fig 1. Analysis of direct and indirect platelet eluates by means of HIV-Western blot immunoassay. Direct and indirect platelet eluates were obtained by ether elution from HIV-infected patient platelets and from normal platelets first incubated with patient sera, respectively, and were analyzed by commercially available HIV1-Western blot kits (Dupont or Diagnostics-Pasteur). According to the manufacturer’s instructions, the markers (left) correspond to the major HIV proteins. Positive control (1), and representative aspects of immunoblots obtained with direct eluates from HIV-infected ITP patients (2-5). Only anti-GP160/120 was observed in 14 of 25 eluates (6,7). Positive immunoblots obtained with direct eluates from HIV-infected nonthrombocytopenic patients. In these cases, negative results were obtained in 7 of 11 eluates (8-10). Pattern of immunoblots obtained with indirect eluates from HIV-infected ITP patients. Only anti-GP160/120 was observed in 12 of 14 indirect eluates (11). The weakly positive immunoblots were obtained with indirect eluates from HIV-infected nonthrombocytopenic patients. Negative results were obtained in six of the eight nonthrombocytopenic patients studied. Direct and indirect eluates of patient Bou (2 and 8, respectively). All direct and indirect eluates were used at a dilution of 1:40 in these experiments.
however, owing to the limited sensitivity of the methods used.

**Binding of serum HIV antibodies to normal platelets.** Because HIV antibodies were present but HIV antigens were not detectable on the membrane of platelets from the HIV-infected ITP patients, we determined whether HIV antibodies could bind to platelets from uninfected healthy blood donors. Indirect platelet eluates were thus obtained by ether elution after incubation of patient sera with normal platelets. As shown in Fig 1, all 14 indirect platelet eluates obtained with serum from HIV-infected ITP patients were positive in the HIV-Western blot immunosay. All of these eluates recognized GP160/120, and two also recognized other viral proteins, ie, p17, p24, p31, p53, p68. In the HIV-ELISA, 13 of these eluates were negative, whereas one (the sample showing antiplatelet activity in the PIIFT, ie, patient Bou [described below]) was strongly positive (four times the cutoff value). In contrast, only two of eight eluates obtained with serum from HIV-infected non-ITP patients were weakly positive in the Western blot immunosay (recognizing GP160/120), whereas all were negative in the HIV-ELISA and the PIIFT. The discrepancies between the results obtained in the HIV Western blot immunosay and the HIV-ELISA with both the indirect and direct eluates may have been due to the presence of only trace amounts of GP160/120 in the ELISA kit, since GP160/120 antibodies were predominant in the eluates. To determine whether this ability of HIV antibodies to bind to platelets from HIV-negative patients was specific (ie, due to the presence of cross-reactive antigens between platelets and virus) or nonspecific (ie, Fc binding), we focused our study on patient Bou, who showed the highest serum titer of antiplatelet and anti-HIV activities able to bind to normal platelets.

**Characterization of PAIgG from patient Bou.** The PAIgG eluted from patient (auto) and normal (allo) platelets preincubated with patient serum were strongly reactive in the PIIFT with platelets from healthy blood group O donors (Table 1). In contrast, they did not recognize ABO-compatible platelets from two GT patients with no detectable GPIIb/IIIa in immunochromatography, strongly suggesting anti-GPIIb and/or anti-GPIIIA specificity of the platelet antibody. Furthermore, the direct Bou platelet eluate immunoprecipitated a band of 97 Kd characterized as GPIIIa (see Fig 3). As we have reported for other patients,2 the sera of the patients in this study could not precipitate platelet GPIIb/IIIa. The direct and indirect eluates also contained HIV antibodies (described above) detectable in the ELISA and HIV-Western blot immunosay (Table 1, Fig 2). In this latter assay, only GP160/120 antibodies were detectable (Fig 1, strips 2 and 8, Table 1). To determine if two distinct antibodies were present in these eluates, we performed absorption/elution experiments with ABO-compatible platelets from two GT patients. Neither the anti-HIV nor antiplatelet activity present in these eluates was present in the patients' sera or in the direct or indirect eluates were able to bind to these GT platelets. These results, contrasting with those obtained in binding/elution experiments with normal healthy blood-donor platelets (described above), suggested that both patient Bou HIV and platelet antibodies bound to GPIIb/IIIa (Fig 2 and Table 1).

The presence of cross-reactive antibody between platelet GPIIb/IIIa and HIV GP160 in patient Bou serum and the anti-GPIIb/IIIa specificity of Bou platelet antibody was confirmed by binding/elution experiments with patient Bou serum on insoluble purified GPIIb/IIIa. As shown in Table 1, the antibodies eluted from purified immobilized GPIIb/IIIa recognized healthy blood-donor ABO-compatible platelets in the PIIFT and HIV-Gp160 in the HIV-Western blot immunosay. The anti-GPIIb/IIIa specificity of the platelet antibody was confirmed by an immunoprecipitation procedure, because, when incubated with 125I-labeled platelets, the eluate obtained from purified immobilized GPIIb/IIIa incubated with patient serum (as well as with direct platelet eluate) precipitated a band of 97 Kd in reducing conditions which comigrated with the GPIIIa precipitated by an PLA1 antibody or a GPIIIa MoAb (SZ21) (Fig 3). Controls of these absorption-elution experiments included serum of a nonthrombocytopenic HIV-infected patient (that did not recognize the immobilized GPIIIb/IIIa) and polyclonal GPIIb/IIIa antibodies from an HIV-negative GT patient.7 (The eluate obtained from the immobilized GPIIb/IIIa column after incubation with this latter serum contained only GPIIb/IIIa antibody).

To determine whether binding of the cross-reactive platelet antibody to normal platelets or to purified GPIIb/IIIa was specific, the same experiments were performed with the F(ab')2 fragment of purified serum Bou IgG. As shown in Fig 2, similar results to those observed with patient serum were obtained with this material. Thus, both the platelet and HIV GP160 antibody activities present in patient Bou F(ab')2 fragments were capable of binding to normal platelets.

### Table 1. Characterization of Patient Bou IgG Platelet Antibody

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patients' Sera</th>
<th>Antibodies Eluted From Patients' Platelets (direct eluate)</th>
<th>Antibodies Eluted From Normal Platelets After Incubation With Patients' Sera (indirect eluate)</th>
<th>Antibodies Eluted From Purified GPIIb/IIIa After Incubation With Patients' Sera</th>
</tr>
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<tbody>
<tr>
<td>IF on normal platelets</td>
<td>+++ to ++++</td>
<td>+++</td>
<td>+ +</td>
<td>+++</td>
</tr>
<tr>
<td>IF on GT platelets</td>
<td>- to +</td>
<td>-</td>
<td>-</td>
<td>ND</td>
</tr>
<tr>
<td>Platelet immunoprecipitation</td>
<td>Negative</td>
<td>GPIIIa</td>
<td>ND</td>
<td>GPIIIa</td>
</tr>
<tr>
<td>HIV-ELISA (cutoff = 0.3 OD)</td>
<td>&gt; 2</td>
<td>1.54 ± 0.30</td>
<td>1.29 ± 0.44</td>
<td>ND</td>
</tr>
<tr>
<td>HIV-Western blot assay</td>
<td>All HIV-proteins and glycoproteins</td>
<td>HIV-GP160/120</td>
<td>HIV-GP160/120</td>
<td>HIV-GP160/120</td>
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Platelet membrane fluorescence was graded as negative (-), weakly positive (+), or positive (+++ to ++++) in comparison with results obtained using a negative control (serum from a non-transfused healthy male blood group AB donor), and a positive control (pool of sera from multitransfused immunized patients).
normal healthy blood donor platelets but not to GT platelets. Likewise, binding/elution experiments with this material on insoluble purified GPIIb/IIIa demonstrated that GPIIb/IIIa antibody was entirely absorbed on the column and that the GPIIb/IIIa column eluate contained HIV GP160 antibody (data not shown). Furthermore, in these experiments, F(ab')2 binding could be demonstrated only with an anti-light chain but not with an antiglobulin directed against the Fc portion of the γ chain, ruling out binding by residual IgG contaminating the F(ab')2 preparation. Overall, these results demonstrated the presence in patient Bou of GP160/120 antibodies able to bind specifically to platelet GPIIb/IIIa.

**Binding of the cross-reactive antibody to r-GP160 of the HIV-1 envelope GP.** To characterize the cross-reactive epitope between HIV-GP160/120 and platelet GPIIb/IIIa, several experiments were conducted (Table 2). Direct Bou platelet eluate did not recognize the cell membrane of HIV-1-infected cell lines (U937-HTLVIll and CEM-Bru), whereas intracytoplasmic staining was observed when infected cells were permeabilized with methanol and Triton X-100, suggesting that the eluate contained HIV antibodies probably directed against the native immature GP160. Binding and elution experiments were performed with patient Bou platelet eluates on the two immobilized r-GP160s of the HIV envelope. Nonabsorbed and eluted materials from the column were tested for anti-HIV and antiplatelet activities. As shown in Table 2, neither the antiplatelet nor anti-HIV activities present in the Bou eluate bound to r-GP160-1, whereas they bound almost completely to r-GP160-2. The same results were obtained with the patient Bou serum F(ab')2 fragments. These results suggest either that the cross-reactive epitope recognized was located on the deleted portion of r-GP160-1 (present on r-GP160-2) or that it is dependent on the glycosylation or conformational structure of the GP.

**DISCUSSION**

Immunologically mediated platelet destruction has been clearly demonstrated in a proportion of HIV-infected patients.6-7,10,12,13,32 The responsibility of immune complex deposition or platelet autoantibody binding in this process is controversial, however. We and other investigators have reported the presence of autoantibodies directed against platelet GPIIb/IIIa and/or other platelet membrane GPs in HIV-infected ITP patients.16-18 On the other hand,
Karpatick et al suggested that both autoantibody fixation and nonspecific immune complex deposition may be responsible for the thrombocytopenia.\textsuperscript{6,12,14} The present findings concerning 28 HIV-infected ITP patients confirm our previous results.\textsuperscript{18} IgG with antplatelet activity was observed in the serum of 89% of the patients. PAIgG levels were increased in 80%, and 9 of 20 direct platelet eluates contained platelet antibodies sometimes immunochemically characterized as anti-GPIIb/IIa. In addition, we noted that in one of these patients (patient Bou), serum antiplatelet IgG specifically bound to normal platelets and purified immobilized GpIIb/IIa. This binding was not explained by the presence of a platelet-bindable antibody that specifically recognizes an epitope (not precisely identified) shared by two GPs. Perhaps only small numbers of HIV-infected patients develop such antibodies, and the presence of both Gp160 and GPIIb/IIa antigens on the platelet membrane, because viral antigens were not detectable in various immunologic tests and especially because the anti-HIV activity also bound to platelets from HIV-negative subjects. The absence of viral antigens or proviral DNA on HIV-ITP platelet extracts has been reported by other investigators.\textsuperscript{14} Furthermore, binding of HIV antibodies to the platelet surface was specific in the case of patient Bou and occurred through the F(ab')\textsubscript{2} portion. Indirect evidence for the responsibility of this anti-HIV activity in immune platelet destruction is the finding that all direct and indirect platelet eluates from thrombocytopenic patients, but only a few of those obtained from nonthrombocytopenic patients, contained anti-GP160/120 antibodies.

The specific binding of both anti-HIV and antplatelet activities to normal platelets and purified GPIIb/IIa suggested the presence of an antibody that recognizes both HIV and platelet antigens. Shared antigenicity between an infectious agent and normal tissue as a mechanism of host destructive activity has been demonstrated in parasitic bacterial and, more recently, viral systems.\textsuperscript{38,41} To test this hypothesis, we focused our study on patient Bou, who showed the highest titer of antplatelet (anti-GPIIb/IIa) and anti-HIV (anti-GP160/120) activities that specifically bound to normal platelets and purified GPIIb/IIa. Results of the absorption-elution experiments on purified immobilized GpIIb/IIa, which bound both patient Bou GPIIb/IIa and HIV GP160 antibody, as well as the absence of binding of either activity to platelets from GT patients clearly demonstrated the presence in the serum or eluates of a single antibody with both activities. This antibody recognized only the native immature GP160 in HIV-infected cell lines. To confirm cross-reactivity, we performed cross-absorption experiments with two r-GP160s and direct and indirect Bou eluates, as well as serum F(ab')\textsubscript{2} fragments. The cross-reactive antibody could be absorbed on r-GP160-2 (expressed in baculovirus-infected insect cells) but not on r-GP160-1 (expressed in vaccinia virus-infected BHK 21 cells). Then, we believe, the absence of recognition of r-GP160-1 would result from abnormal glycosylation leading to conformational changes and rendering some epitopes inaccessible.

Therefore, we clearly demonstrated in patient Bou the presence of a platelet-bindable antibody that specifically recognizes an epitope (not precisely identified) shared by HIV GP160/120 and platelet GPIIb/IIa, suggesting that homologies exist between the two GPs. Perhaps only small numbers of HIV-infected patients develop such antibodies.\textsuperscript{40,41} However, the presence of both GP160 and GPIIb/IIa antibodies in at least 5 of the 20 direct platelet eluates studied argues for the frequent responsibility of cross-reactive antibodies in the immune immune platelet destruction observed in HIV-infected patients. Finally, if the hypothesis of cross-reactivity is confirmed, the antigenic structure involved will be of importance because it will have to be taken into account in treatment of such patients and in production of vaccines based on HIV envelope components.

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Presence of cross-reactive antibody between human immunodeficiency virus (HIV) and platelet glycoproteins in HIV-related immune thrombocytopenic purpura

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