Clonal Restriction of Platelet-associated Anti-GPIIb/IIIa Autoantibodies in Patients with Chronic ITP

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Key words

ITP, autoantibody, platelet, clonality

Summary

Chronic immune thrombocytopenic purpura is due to platelet destruction induced by autoantibodies against platelet surface antigens. Prior studies show that some serum autoantibodies are light-chain restricted, suggesting a clonal origin. Since plasma and platelet-associated antibody from the same patient may bind to different epitopes, it is important to evaluate the clonality of platelet-associated antibody. Platelet-associated autoantibodies from 28 ITP patients were studied. Of 23 platelet-associated antibodies tested directly, 16 showed significant light chain restriction (7 complete and 9 partial) when compared to plasma IgG light chain distribution. Similarly, 9 of 12 platelet-associated antibody eluates were light chain restricted, 5 complete and 4 partial. In all cases where platelet-associated antibody and antibody eluate from the same patient were studied, the results were concordant. We conclude that a significant proportion of platelet-associated antibodies from ITP patients appear apparent clonality, as evaluated by light chain restriction. These results are consistent with other studies in ITP suggesting a limited antigenic repertoire.

Introduction

Chronic immune thrombocytopenic purpura (ITP) is manifested by autoantibody-induced platelet destruction due to antibodies against platelet surface proteins, usually glycoprotein (GP) IIb/IIIa or Ib/IX (1-3). Older platelet-associated IgG studies suggested autoantibody polyclonality (4-6). That conclusion is suspect because: (a) animal studies show that a single antibody-producing clone can generate progeny of different heavy chain classes while maintaining the same light chain and epitope specificity (7) and (b) most PAIgG is not anti-platelet antibody (8, 9).

More recent reports suggest that antibodies in some patients with immune thrombocytopenia, including ITP, may be clonal in origin (Table 1). Thus far, clonality studies in chronic ITP have been limited to the evaluation of serum antibodies. Since serum and platelet-associated autoantibodies (PAAb) from the same ITP patient may bind to different epitopes (10), PAAb studies are warranted since these antibodies are more likely involved in disease pathogenesis.

Materials and Methods

Patients. Platelet-associated antibody against GPIIb/IIIa from 28 ITP patients was studied: PAAb and eluted PAAb- 7 pts; PAAb only- 16 pts; eluted PAAb only- 5 pts.

Evaluation of Clonality. We determined the relative distribution of IgG autoantibodies with kappa or lambda light chains.

Platelet lysate and eluate preparation. Patient or control platelets were washed 6 times with 0.05 M isotonic citrate buffer, pH 6.2 and solubilized in 1% Triton-X100. Autoantibody eluates were prepared by acid elution, as previously described (10).

Kappa and lambda chain percentages of PAAb. Microtiter wells (Dynex Technologies, Middlesex, UK) were coated for 2 h at RT with 200 μl of monoclonal anti-GPIIb (2A9, provided by Dr. Virgil Woods, UCSD) at 10 μg/ml in 0.1 M NaHCO3 buffer, pH 8.4, followed by blocking with 2% BSA. After 6 washes with 0.05% Tween-20 in PBS, 200 μl of either patient or control platelet lysate (108 plts/ml) were added. After overnight incubation at 4 C and 6 washes, bound IgG kappa or lambda chains were detected by serial 60 min incubations (followed by washing) with 200 μl of either biotinylated goat anti-human kappa or lambda chains (Pierce, Rockford, IL), followed by preformed avidin/ biotinylated horseradish peroxidase complexes and o-phenylenediamine dihydrochloride substrate (ABC System, Vector Laboratories, Burlingame, CA). Net optical density (mean patient OD-mean control OD) was determined and the relative kappa and lambda chain percentages were calculated.

Kappa and lambda chain percentages of eluted PAAb. Microtiter wells were coated overnight at 4 C with 100 μl of monoclonal anti-GPIIb and blocked with 2% BSA. After washing, 100 μl of either platelet lysate (source of GPIIb/IIIa) or 2% BSA (control) were added to selected wells and incubated for one hour. Following washing, 100 μl of patient eluate were added to wells containing either platelet lysate or BSA. After 2 h incubation and 6 washes, bound IgG kappa or lambda chains were detected and their relative percentages calculated as described above.

Plasma kappa and lambda chain percentages. Microtiter wells were coated for 2 h with 50 μl of monoclonal anti-human IgG (HB-43 American type Culture Collection, Rockville, MD) or anti-GPIIb, as a control, and blocked with 5% milk protein. Next, 50 μl of serial 1:2 dilutions of plasma from ITP patients or control subjects, beginning at 1/10,000, were added to wells containing either anti-IgG or control antibody. After 2 h incubation and 6 washes, light chains were detected as above. Net optical densities were plotted against plasma dilutions and relative kappa and lambda optical densities were calculated from the linear portion of of the curves.

Adsorption of antibody eluates with immobilized anti-light chain antibodies. Selected eluates (ITP-7 and ITP-9) were preincubated with biotinylated murine monoclonal anti-human kappa chain or anti-human lambda chain antibody linked to streptavidin beads to remove kappa or lambda-chain autoantibodies from the eluate prior to testing. After overnight incubation of 250 μl of streptavidin beads (1-2 mg streptavidin/ml, Pierce, Rockford IL) with 600 μl of PBS containing 40 μl (0.5 mg/ml) of goat anti-human kappa or lambda chain

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antibody, the beads were centrifuged, washed with PBS and resuspended in 250 μl of PBS. To 100 μl of antibody eluate were added 40 μl of the bead suspension from either the anti-kappa or anti-lambda beads or beads without bound antibody, as a control. After overnight incubation at 4 °C, an additional 100 μl of PBS was added to each tube and after centrifugation the adsorbed eluates were tested for residual antibody.

Results

Clinical Features

We studied 28 patients with chronic ITP, 16 women and 12 men with ages ranging from 20 to 79. Ten patients had other autoimmune disorders: thyroid disease- 5; antiphospholipid antibodies- 2; SLE- 3; autoimmune hemolytic anemia and immune neutropenia- one each. Twenty-one of 26 patients had undergone splenectomy (in 2, the splenic status was not known) and none had responded. All patients had elevated levels of platelet-associated autoantibody to GPIIb/IIIa and 6 also had autoantibody to GPIb/IX. In addition, we studied plasma from 10 control subjects, 11 ITP patients and a sample of pooled human IgG (Sandoglobulin, Novartis Pharmaceuticals Corp.).

Plasma Results

There were no significant differences between normal plasma kappa and lambda chain results (κ/λ percentages: 59.2 ± 3.8 and 40.8 ± 3.8, respectively; mean κ/λ chain ratio of 1.47 ± 0.23) and ITP plasma kappa and lambda results (κ/λ percentages: 56.4 ± 1.8 and 43.6 ± 1.8, respectively; mean κ/λ ratio of 1.3 ± 0.1). Pooled IgG had a ratio of 1.66.

Autoantibody Results

Platelet-associated antibody. Of the 23 PAAbs tested (Fig. 1), 7 showed complete light chain restriction, 6 kappa (ITP- 3, 7, 9, 19, 22 and 26) and one lambda chain (ITP-23); nine others showed partial light chain restriction (>3 SD above the mean of normal plasmas), 7 kappa (ITP-8, 11, 14, 16, 17, 18 and 24) and two lambda (ITP- 4 and 12).

Autoantibody eluates. Nine of 12 PAAb eluates were light chain restricted (data not shown): 5 were complete, 4 kappa (ITP- 3, 7, 11 and 25) and one lambda (ITP-2) and 4 were partial, all kappa (ITP-9, 10, 13 and 27). In all cases where platelet-associated antibody and antibody eluate from the same patient were studied, clonal restriction involved the same light chain.

Adsorption Studies

Two kappa light chain-restricted eluates (ITP-7 and ITP-13) were tested with and without adsorption by immobilized anti-kappa and anti-lambda antibody. In both cases, adsorption with anti-kappa

Table 1 Previous antibody clonality studies in patients with immune thrombocytopenia

<table>
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<tr>
<th>Study (Year)</th>
<th>Results</th>
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<tr>
<td>Christie et al, 1993 (13)</td>
<td>Noted B Cell clonality and partial or complete light chain restriction in 18 of 21 serum alloantibodies or drug-dependent antibodies.</td>
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Fig. 1 Kappa and lambda light chains in platelet-associated autoantibody. The two horizontal dashed lines represent 3 standard deviations above the mean percentage of kappa and lambda chains in normal plasma.
antibody resulted in >86% removal of the autoantibody activity (Table 2).

**Discussion**

It has been previously reported that serum antibodies from many ITP patients (11, 12), as well as patients with other types of immune thrombocytopenia (13), are light chain restricted suggesting antibody clonality. Since only serum antibodies were studied, it was important to confirm these findings using PAAb which are more likely to be involved in the disease pathogenesis.

The present studies show that PAAb from 20 of 28 patients with chronic ITP are either completely or partially light chain restricted, adding further support for autoantibody clonality in chronic ITP. The presence of autoantibody clonality in ITP is consistent with several previous observations suggesting that ITP patients have a limited antigenic repertoire (14-18).

There are other possible interpretations of these results. Some studies of human monoclonal antibodies, obtained using EBV or combinatorial technology, show partial or complete light chain restriction despite DNA sequencing showing that the antibodies differ genetically (19, 20). This suggests that certain epitopes may require either kappa or lambda light chains for epitope recognition. Whether human monoclonal antibodies, which are produced in vitro, accurately reflect the in vivo situation is unknown. The monoclonal antibody systems have the advantage of being able to genetically characterize the heavy and light chain which are produced but the disadvantage of not knowing if the cloned monoclonal antibodies are the same as those of the patient. Conversely, we have some assurance that the platelet-associated antibodies, which we studied, are those involved with the disease pathogenesis but we are unable to evaluate them genetically. It would seem that information obtained with both approaches is useful, particularly when data concerning monoclonal antiplatelet antibodies, derived from ITP patients, become available.

In summary, these results showing light chain restriction of platelet-associated antibodies from ITP patients are consistent with antibody clonality and support the previous findings with serum antibodies and the observations which show that the antigenic repertoire in chronic ITP is limited.

**References**


