Specificity of Autoantibodies in Autoimmune Thrombocytopenia

By E. F. van Leeuwen, J. Th. M. van der Ven, C. P. Engelfriet, and A. E. G. Kr. von dem Borne

In 42 patients with autoimmune thrombocytopenia (AITP) and a positive direct platelet suspension immunofluorescence test (PSIFT), the antigenic specificity of the autoantibodies was studied. Because the autoantibodies were often not detectable in the serum and additional HLA antibodies may disturb the reaction pattern with the platelet panel, we used eluates prepared from the patients’ platelets for this study. Thirty-five patients had antibodies equally reactive with normal platelets, irrespective of their antigenic make-up, but not with the platelets from two Glanzmann’s disease patients. Absorption and elution experiments in two patients showed that this was probably not due to the presence of a combination of anti-Zw* and anti-Zw* antibodies. Thus, the majority of autoantibodies against platelets seems to be directed against antigenic determinants not present on Glanzmann’s disease platelets, but perhaps located on the platelet-membrane glycoproteins IIb and/or IIIa. In ten patients, antibodies of no, or still unknown, specificity were detected. Three of these had additional antibodies not reactive with the platelets of the two Glanzmann patients.

In autoimmune hemolytic anemia, the responsible autoantibodies frequently have blood group specificity. The majority of the antibodies (about 70%) are directed against an antigen or antigens of the rhesus system. This is most evident when the antibodies are tested with rhesus-null cells.1 The specificity of the autoantibodies in autoimmune thrombocytopenia (AITP) is unknown. This is mainly because, until recently, no simple and reliable serologic test was available for the detection and characterization of platelet autoantibodies. With the newly developed platelet immunofluorescence test,2 we detected autoantibodies on the platelets of all of them.

In this article, we describe the results of our study in 42 patients with AITP.

MATERIALS AND METHODS

Patients

The 42 patients with thrombocytopenia selected for this study all fulfilled the diagnostic criteria for ITP, as described previously.3 Moreover, with the platelet suspension immunofluorescence test (PSIFT), we detected autoantibodies on the platelets of all of them and in an eluate prepared from their platelets.

Techniques

Blood samples, anticoagulated with EDTA, and sera from blood clotted at room temperature were used. The direct PSIFT was performed on the patient’s own platelets, as described in detail elsewhere.4 The indirect PSIFT was done by incubating the sera of the patients with the platelets from random healthy donors of blood group O. Positively reacting sera (19 of 42) were screened for the presence of HLA antibodies with the standard NIH lymphocyte microcytotoxicity test with the lymphocytes of the donors of our platelet-typing panel (see below). Only sera that gave negative reactions with these lymphocytes (14/19) were used in this study. The presence of EDTA-dependent antibodies in these sera was excluded by testing them with citrate platelets. These antibodies, present in sera from patients with pseudothrombocytopenia, may give a false-positive PSIFT in the presence of EDTA.

Ether eluates were prepared from the platelets of the patients with diethylether, as previously described. All the eluates gave positive reactions with random donor platelets in the PSIFT. The eluates were kept at -20°C until they were tested with the platelets of our typing panel in the PSIFT. The platelets of this panel were from healthy donors of group O, selected for their phenotype for the platelet-specific alloantigens Zw*(P1A1), Zw* (P1A2), Ko*, Ko, and Bak* (A–F in Table 1), determined as previously described.5 The elutes and sera were tested with the platelets of at least 8 normal donors, which included all these phenotypes. Eluates and sera were also tested with the platelets of two patients with Glanzmann’s thrombasthenia (GT) (phenotype G). Analysis of the membrane glycoproteins of these GT platelets showed a marked reduction of glycoprotein IIb and IIIa, and the Zw antigens proved to be completely deleted.6 Both patients were of GT type I. Four sera were also tested with the platelets of two patients with the Bernard-Soulier syndrome. They fulfilled the diagnostic criteria of this disease: prolonged bleeding time, moderate thrombocytopenia, giant platelets, reduced platelet-subendothelial adhesion, no aggregation in response to bovine factor VIII, but normal aggregation on ADP and collagen. Analysis of the platelet membranes in both patients by SDS-PAGE showed a marked reduction of GP Ib and Ia, as described in the literature.8 The expression of the known platelet-
specific alloantigens proved to be normal in these patients (unpublished results).

The platelets of the panel were stored by cryopreservation in liquid nitrogen with 5% DMSO, as described for lymphocytes. Shortly before use, they were thawed in AB serum plus 10% EDTA and washed three times. The antigenic expression on the platelets after freezing and thawing by this method was unchanged, as measured cytofluorometrically.

In seven cases, only enough eluate was available to test it once against the panel. Therefore, a polyspecific anti-human immunoglobulin reagent, labeled with FITC (prepared in our laboratory), was used in the PSIFT, even when it was known that IgG and IgM antibodies were present in the eluate. In all other cases, monospecific anti-IgG, anti-IgM, and/or anti-IgA reagents, prepared by Dakopatts, Copenhagen, Denmark, were used.

In the absorption and elution experiments, 1 ml of serum or eluate was absorbed twice with 60 x 10^6 platelets of Zw(a^+ b^-) donors for 1 hr at 37°C. The mixture was centrifuged and the supernatant was removed. Elution of the antibodies from the platelets after absorption was then performed not by ether treatment but by heating the platelets, suspended in 1 ml PBS 1% BSA, pH 7.2, for 60 min at 56°C. This was done simply as a matter of convenience. A pilot study showed that elution with ether or by heating gave the same results under the conditions used for these experiments.

The serum (after absorption) and the eluate were tested in dilutions against the platelets from two Zw(a^+ b^-) donors. The same procedure was performed using Zw(a^- b^+) donors. The same procedure was performed using Zw(a^- b^+) platelets and platelets from patients with GT as absorbing cells.

**RESULTS**

The eluates prepared from the platelets of 19 patients with IgG autoantibodies were equally reactive with all normal donor platelets of the panel, irrespective of their antigenic make-up. Eleven of these eluates showed no reaction with the platelets of both patients with GT. Eight gave fluorescence of the same intensity, both with normal and with thrombascenic platelets (see Table 2). In one of the 11 patients, the eluate of the platelets obtained during the first episode of thrombocytopenia reacted only with the normal donor platelets of the panel and not with the GT platelets, whereas an eluate prepared from the platelets during a second exacerbation of the disease, reacted equally with the platelets of both GT patients.

Eluates from the platelets of one patient with IgA autoantibodies and five patients with IgM autoantibodies also reacted equally well with all normal plate-
for the absorption, reacted equally strongly with Zw(a + b−) and with Zw(a − b+) platelets. The same results were obtained when this absorption procedure was performed with Zw(a − b+) platelets from another donor. The results of this experiment with eluate B are depicted in Table 3. With eluate A, identical results were obtained.

The eluates from the platelets of two other patients, both reactive with normal and GT platelets, were absorbed with GT platelets. After absorption, one eluate showed no reaction with normal or with GT platelets, whereas the eluate prepared from the GT platelets after absorption reacted equally well with both normal and GT platelets. The other eluate still showed a positive reaction with normal platelets after absorption, although more weakly than before absorption, but not with GT platelets. The eluate prepared from the GT platelets after absorption reacted with normal with and GT platelets. These results are depicted in Table 4. Thus, the second eluate probably contained two antibody populations, one reactive and one not reactive with GT platelets.

**DISCUSSION**

The specificity of autoantibodies against platelets is not yet known. Survival studies of radioactively labeled platelets in patients with idiopathic thrombocytopenic purpura showed no difference when either random donor platelets or autologous platelets were used. This indicates that the antibodies behave as "panantibodies," reacting with all human platelets.

Karpatkin et al., using the platelet-factor-3 immune-injury technique, found panreactivity of the antibodies in the serum of ITP patients, although in some patients a degree of individual specificity was seen. However, studies by Donnell et al., who used the Fab-anti-Fab IgG assay system, seem to contradict these results.

**Table 3. Absorption-Elution Experiments With an Eluate B Containing Autoantibodies Against Platelets Reactive With Normal Platelets, But Not With Platelets From Patients With Glanzmann’s Thrombasthenia (in Titers†)***

<table>
<thead>
<tr>
<th>Serum before absorption</th>
<th>Zw(a + b−) Platelets</th>
<th>Zw(a − b+) Platelets</th>
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</thead>
<tbody>
<tr>
<td>Serum after absorption with Zw(a + b−) platelets</td>
<td>—</td>
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<tr>
<td>Eluate prepared from absorbing Zw(a + b−) platelets</td>
<td>1 : 4</td>
<td>1 : 4</td>
</tr>
<tr>
<td>Serum after absorption with Zw(a − b+) platelets</td>
<td>—</td>
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<tr>
<td>Eluate prepared from absorbing Zw(a − b+) platelets</td>
<td>1 : 4</td>
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*Highest dilution in which antibodies were demonstrable.

We applied the PSIFT and found that in 42 patients with AITP, the autoantibodies present in the eluates or the serum were equally reactive with all normal platelets, irrespective of the phenotype of the platelet donors, thus confirming the work of Karpatkin et al. However, in most patients (35/42), the autoantibodies did not react with the platelets from 2 patients with GT. This result is in contrast with that of Kunicki et al., who used the 51Cr-lysis assay and found a normal reaction with the platelets from patients with GT. In that study, sera from only two patients with AITP were included, and it is possible that negative results would have been found if more sera had been tested.

In GT, there is a marked reduction of the membrane glycoproteins IIb and IIIa. Thus, our findings seem to indicate that in most patients with AITP, the autoantibodies are directed against antigenic determinants carried by these glycoproteins. Recently, it was demonstrated that the alloantigens of the Zw system are deleted from the platelets of patients with GT. and Kunicki et al. have reported that glycoprotein IIb carries the antigenic determinants of Zw. Therefore, it could be that the reactivity with normal platelets and not with GT platelets is due to the presence of a combination of anti-Zwa and anti-Zwb antibodies. However, the antibodies reacted equally well with all normal donor platelets, independent of their Zw phenotype. This was also so when tested in titration and was confirmed by absorption and elution experiments. Thus, the antibodies were not directed against the Zw and/or Zww determinants on these glycoproteins, but against determinants independent of these Zw antigens. It is likely that these antigens are located on GP IIb and/or IIa, although a location on other glycoproteins cannot be excluded presently. Immunoprecipitation studies are necessary to solve this problem.

In 10 of the 42 patients, the autoantibodies appeared to have no, or still unknown, specificity. In 3 of these 10 patients, two different reaction patterns were found. In 2 patients, the antibodies in the serum did not react with GT platelets, whereas the antibodies...
in the eluate did. It is likely that in these patients two different antibody populations were present, one totally absorbed by the patient’s platelets with no, or unknown, specificity, and another, also present in serum, directed against antigenic determinants absent from GT platelets. In the third patient, absorption of the eluate with GT platelets showed that the autoantibodies against platelets without apparent specificity comprised the above mixture of antibodies. This is a reflection of the situation in autoimmune hemolytic anemia, in which a mixture of autoantibodies, reactive and nonreactive with Rh-deleted red cells, may be present.

In conclusion, we found that in most patients (about 80%) with autoimmune thrombocytopenia and a positive direct PSIFT, the antibodies are directed against antigenic determinants lacking from GT platelets, probably carried by the membrane glycoproteins IIb and/or IIIa. Therefore, it will be of interest to study platelet function in these patients to see whether autosensitization of platelets in vivo can induce a Glanzmann-like state.

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