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Platelet-Associated and Plasma Anti-Glycoprotein Autoantibodies in Chronic ITP

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Chronic immune thrombocytopenic purpura (ITP) is due to platelet destruction by circulating antiplatelet antibody. Although autoantibodies against the platelet glycoprotein IIb/IIIa (GPIIb/IIIa) complex and GPIb have been demonstrated using various methods, practical assays for detection of platelet-associated or plasma autoantibodies have not been available. We studied 59 patients with chronic immune thrombocytopenic purpura in whom platelet-associated and plasma autoantibodies against the GPIIb/IIIa complex and GPIb were measured using a newly developed immunobead assay and a previously reported microtiter-well assay. Platelet-associated autoantibody was detected using the immunobead assay in 21 of 28 patients (75.0%; 13 with anti-GPIIb/IIIa, 8 with anti-GPIb). Plasma autoantibodies were noted in 34 of 59 patients (57.6%; 21 with anti-GPIIb/IIIa, 11 with anti-GPIb, and 2 with both). Positive results were noted in 30 of 59 patients using the immunobead assay and in only 14 of 59 using the microtiter-well assay, suggesting that solubilization of the platelets prior to antibody addition, as in the microtiter-well assay, alters epitope stability. Of the 31 thrombocytopenic control patients studied, all gave negative results using both assays. We conclude that these clinically adaptable assays allow detection of autoantibodies in most patients with chronic ITP, confirming the presence of an autoimmune process.

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CHRONIC IMMUNE thrombocytopenic purpura (ITP) is a syndrome of destructive thrombocytopenia due to an antibody against a platelet-associated antigen.[1,2] Van Leeuwen et al[3] provided the first evidence for autoantibodies in ITP patients when they noted that 32 of 42 antibody eluates from ITP platelets would bind to normal but not to thrombasthenic platelets. Because thrombasthenic platelets are deficient in platelet glycoproteins (GP) IIb and IIIa, they suggested that these ITP patients had autoantibodies to one of these GPs. Direct evidence for anti-GP autoantibodies in chronic ITP has been provided by subsequent studies. Woods et al showed binding of autoantibodies from ITP patients to the GPIIb/IIIa complex or to GPIb attached to microtiter wells with monoclonal antibodies and confirmed these observations by immunoprecipitation.[4,5] They noted anti-GPIIb/IIIa or anti-GPIb autoantibodies in ~10% of patients, much less than the percentage observed by the indirect studies of van Leeuwen et al.[3] Other investigators also detected autoantibodies in chronic ITP patients using immunoblotting,[6,7] immunoprecipitation,[4,5,8] inhibition of murine monoclonal anti-GPIIb/IIIa antibody binding to ITP platelets,[9] and crossed immunoelectrophoresis.[10] Nugent et al[11] and Asano et al[12] established human hybridomas from ITP lymphocytes that synthesize monoclonal antiplatelet antibodies, some of which are specific for platelet GPs.[11]

Of the assays used for demonstrating anti-GP autoantibodies in chronic ITP, the microtiter-well assay[4,5] is most easily adaptable to clinical use. The low percentage of positive tests (~10%), however, when compared with that of van Leeuwen et al (~76%) suggested to us that solubilization of the platelets prior to antibody sensitization may alter some of the epitopes. For this reason, we designed an assay (immunobead assay) for anti-GP autoantibodies where platelets are sensitized prior to their solubilization, postulating that epitopes may remain more stable when bound to antibody. This assay can measure both platelet-associated and plasma autoantibodies. We studied 59 chronic ITP patients and noted platelet-associated autoantibodies in 21 of the 28 patients and plasma autoantibodies in 34 of 59 patients.

MATERIALS AND METHODS

Study subjects. We studied plasma or platelet samples obtained between January 1, 1982, and September 1, 1986, from 59 patients with chronic ITP, 20 control subjects, and 31 thrombocytopenic controls [acute nonlymphocytic leukemia, 5 patients; acute lymphoblastic leukemia, 2 patients; bone marrow transplantation patients with (4 patients) and without (2 patients) anti-HLA antibodies; aplastic anemia, 2 patients; non-Hodgkins lymphoma, 4 patients (one with cryoglobulins); cirrhosis, 1 patient; myeloproliferative disease, 4 patients; carcinoma on chemotherapy, 3 patients; systemic lupus erythematosus with thrombocytopenia, 3 patients; and immune neutropenia, 1 patient].

Patients with ITP were thrombocytopenic with normal or increased numbers of megakaryocytes and without evidence of other types of immune thrombocytopenia. Platelet-associated and plasma autoantibodies were both studied in 28 of 30 patients seen after March 1986 when the immunobead assay first became available; in 2 of these patients, we could not obtain sufficient platelets for study. Prior to March 1986, only plasma samples were available for assay. The majority of patients (46 of 59) were seen and studied at our institution; the remainder were seen by physicians in the San Diego area (5 patients) or in other parts of the country (8 patients), and samples were referred to us for study. The patient group is biased toward patients with more severe disease as reflected by the large number (24 patients) who were first studied after having failed to attain a remission with splenectomy.

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In all studies in this report, EDTA-anticoagulated blood was studied. Preliminary studies have shown that similar results can be obtained with either ACD-A–anticoagulated blood or serums although serum tends to give higher background values in the microtiter well assay.

Assays for anti-GP autoantibody. The specificity of both the immunobead and microtiter assays is determined by the monoclonal anti-GP antibody used. Both assays are shown schematically in Fig 1. The following murine monoclonal antibodies were used: anti-GPIIb/IIIa-2A9, 3F5, 2G12—2A9 is specific for GPIIb, and the others are complex-specific; anti-GPIb-P3 (provided by Drs Zaverio Ruggeri and Theodore Zimmerman, Scripps Clinic); and anti-human IgG (American Type Culture Collection, Rockville, MD, ATCC HB-43). Monoclonal antibodies (50 μg) were labeled with 500 μCi of ^{125}I using the chloramine-T method. All incubations in both assays were carried out at room temperature.

In detergent extracts GPIIb and GPIIIa are known to form a complex and GPIb is known to be complexed with platelet GP IX. Therefore, as measured by these two assays, anti-GPIIb/IIIa autoantibodies could be against epitopes on either GPIIb or GPIIIa and anti-GPIb specific for GPIb or GP IX. For the purposes of this report, however, results are reported as either anti-GPIIb/IIIa or anti-GPIb, referring to autoantibodies against proteins of the GPIIb/IIIa complex or the GPIb complex.

Immunobead assay. The immunobead assay can be used to measure either platelet-associated autoantibody or plasma autoantibody.

Immunobead preparation. Anti-IgG–coated immunobeads were prepared by incubating polystyrene beads (Poly-Sep, Polysciences, Warrington, PA) with murine monoclonal anti-human IgG (ATCC HB-43) in 0.1M $NaHCO_3$, pH 8.5 for 60 minutes at a bead/antibody ratio of 2,000:1 by weight (eg, beads 100 mg/50 μg of anti-IgG in 2 mL saline). The beads were then centrifuged for 10 seconds at maximum buffer speed in a tabletop centrifuge (International Clinical Centrifuge, Boston, Mass, Model 54133M). After being washed once with 10 mL 0.05% Tween-20 in phosphate-buffered saline, pH 7.4 (PBS-Tween), nonspecific binding sites were blocked by incubation of the beads in 2% bovine serum albumin (BSA) in PBS-Tween for 60 minutes, followed by four washes in PBS-Tween. For recent assay improvements, see "note added in proof" at the end of the manuscript.

Platelet preparation. Platelets from EDTA-anticoagulated blood were obtained from the patient or from a normal donor and washed six times with 0.05 mol/L isotonic citrate buffer.[13] For preparation of antibody-sensitized platelets, washed normal platelets (10^8 in 0.1 mL) were incubated with 900 μL of patient or control plasma, containing PGE_1 (1 μg/mL) and theophylline (1 μmol/L), for 60 minutes at room temperature and then washed four times with 0.05 mol/L citrate buffer containing PGE_1 and theophylline. Patient platelets (10^8) or the antibody-sensitized platelets were resuspended in 900 μL citrate buffer containing leupeptin (100 μg/mL) and then solubilized by the addition of 100 μL of 10% Triton X-100. Control samples were handled similarly.

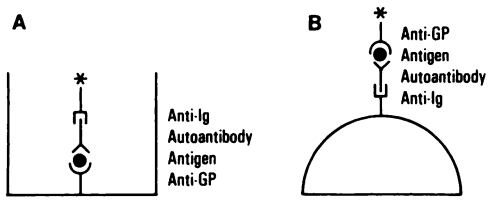

Fig 1. Antiplatelet autoantibody assays. (A) Microtiter-well assay; (B) immunobead assay.

Assay. The solubilized platelets from each sample were centrifuged at 12,000 g for 5 minutes. Preliminary studies showed that this step was required, particularly in anti-GPIb autoantibody studies, to prevent falsely elevated values. Nine hundred microliters of supernate was then incubated for 60 minutes with 100 mg of anti-IgG–coated immunobeads to allow attachment of IgG and any bound antigen. After four washes with PBS-Tween, the presence of specific antigen was demonstrated by incubating the beads with 1.0 mL PBS-Tween containing ~400,000 cpm of ^{125}I monoclonal antibody specific for either anti-GPIIb/IIIa (a cocktail of three monoclonal antibodies specific for noncompeting sites, 2A9, 3F5, and 2G12) or anti-GPIb (P_3) for 60 minutes at room temperature and then washing them four times with PBS-Tween. The beads were resuspended in 1 mL buffer, and 0.5 mL was removed for determining radioactivity. Data are expressed as a binding ratio of cpm of patient sample/mean cpm of three control samples. The mean percentage of variation results of replicate samples of control platelets and platelets sensitized with control plasma were anti-GPIIb/IIIa- 10.1 ± 7.5 (14 studies) and 9.1 ± 8.1 (31 studies), respectively, and anti-GPIb- 6.8 ± 7.0 (17 studies) and 7.9 ± 7.6 (24 studies), respectively. Patient samples with a binding ratio of >1.3 are considered positive (>2 SD over control). The radioactivity bound to control beads ranged from 500 to 1,200 cpm depending on the activity of the labeled monoclonal antibody and the time from labeling to its use. Antibodies were freshly labeled at least every 4 weeks. Preliminary studies show that positive reactivity can be removed by adsorption of plasma with excess platelets. Storage of samples for up to 4 days at 4°C did not affect platelet or plasma control values.

Microtiter-well assay. Details have been previously published.[4,5] In brief, washed platelets (10^9 mL) or CEM leukemic cells (10^7 mL) in PBS containing leupeptin (100 μg/mL) were solubilized in 1% Triton X-100 for 30 minutes at 4°C and then ultracentrifuged (100,000 g for 60 minutes). The lysates were stored at −70°C. Microtiter wells were coated overnight at 5°C with 100 μL of either anti-GPIIb/IIIa (2A9 or 3F5) or anti-GPIb (P3) at a concentration of 5 μg/mL. After six washes with 200 μL PBS-Tween, the remaining binding sites were blocked for 60 minutes with 200 μL 2% BSA in PBS-Tween. After six washes with PBS-Tween, 100 μL of platelet lysate or the antigen negative CEM lysate, diluted 1:10, was added and incubated for 60 minutes. This allows attachment of the specific platelet antigen to the well-bound monoclonal antibody. After six washes, appropriate dilutions (1:10 for screening plasma and higher dilutions if positive) of patient or control plasma were added and incubated for 60 minutes. After six washes, 100 μL of radiolabeled murine monoclonal antihuman IgG (~100,000 cpm) was added and, after 60-min incubation and six final washes, the radioactivity of each well was determined. The percentage of variation for replicate control plasmas is −5.0 ± 7.7 for anti-GPIIb/IIIa and −1.9 ± 6.4 for anti-GPIb.[4,5] Samples with a percentage of increase of >11 were considered positive (>2 SD).

RESULTS

Chronic ITP patients. The results in all patients are summarized in Table 1. The data on individual patients are also analyzed in terms of autoantibody patterns: group 1, 10 patients who were positive in both the immunobead (platelet-associated or plasma antibodies) and microtiter-well assays (Table 2); group 2, 26 patients who were positive in the immunobead assay (platelet-associated or plasma antibodies) but negative in the microtiter-well assay (Table 3); group 3, 4 patients who were positive in the microtiter-well assay but negative in the immunobead assay (Table 4); and

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Table 1. Summary of Antiplatelet Glycoprotein Autoantibody Results

	Entire Group	Assay Pattern Group* 1	2	3	4
No. of patients	59	10	26	4	19
Platelet-associated antibody					
Total no. studied	28	5	16	1	6
No. positive	21 (75.0%)	5	16	0	0
Anti-GPIIb/IIIa	13	2	11	—	—
Anti-GPIb	8	3	5	—	—
Plasma antibody					
Total no. studied	59	10	26	4	19
No. positive	34 (57.6%)	10	20	4	0
Anti-GPIIb/IIIa	21	4	16	1	—
Anti-GPIb	11	5	4	2	—
Both	2	1	0	1	—
Splenectomy results					
No. of surgeries	44	8	20	3	13
Complete remissions	12	0	6	1	5

*Group 1, positive in both assays; group 2, positive in only the immunobead assay; group 3, positive in only the microtiter-well assay; and group 4, negative in both assays.

group 4, 20 patients who were negative in both assays (Table 5). It was not possible to correlate the degree of autoantibody positivity with the severity of the patient's disease, since samples were obtained at different times during the patient's disease and when they were receiving different types of treatment.

Platelet-associated autoantibody. Platelet-associated autoantibody was measured using the immunobead assay in 28 patients (Tables 2 and 3). Of these, 21 (75.0%) were positive, with ratios ranging from 84.5 to 2.2 (control values <1.3); 19 of the 21 patients had ratios of ≥ 5.0, with a mean ratio of 18.6. Thirteen patients had anti-GPIIb/IIIa, and eight had anti-GPIb autoantibodies. In six patients, only platelet-associated autoantibodies were detected and the patients had negative plasma assays.

Platelet-associated autoantibodies in three patients (patients 24, 26, and 29) were studied before and after splenectomy. In the patient who attained a complete remission (patient 29), the anti-GPIIb/IIIa autoantibodies were no longer present after splenectomy. In the two others, the autoantibodies persisted despite splenectomy and although both obtained a temporary increase in the platelet count after surgery (135,000/μL in patient 24 and 313,000/μL in patient 26), they both became severely thrombocytopenic (<20,000) within a few weeks after surgery.

Plasma autoantibody. Plasma autoantibodies were studied in 59 patients using both the immunobead (58 patients) and microtiter-well assay (59 patients). Thirty-four patients (57.6%) were positive; 10 were positive against the same antigen with both assays (Table 2), and 20 were positive with the immunobead assay alone (Table 3). Four samples were positive with the microtiter-well assay alone (Table 4). Of the 34 positive samples, 21 patients had anti-GPIIb/IIIa autoantibodies, 11 had anti-GPIb autoantibodies, and 2 had both types (patient 6 and patient 32).

Autoantibody results and splenectomy. Because many of our patients were studied for the first time after failing splenectomy (24 patients), we believed that these results might not reflect the true incidence of positive tests in newly diagnosed patients. For this reason, we compared the results obtained from patients studied initially prior to surgery with those from patients studied for the first time after failing splenectomy. Neither the number of positive platelet-associated autoantibodies (12 of 17 preoperatively; 9 of 11 postoperatively) nor the number of positive plasma autoantibodies (19 of 32 preoperatively; 15 of 21 postoperatively) differed significantly between the two groups. The number of patients testing positive for both assays was much greater in those studied first after surgery (8 of 15 postoperatively; 1 of 17 preoperatively), whereas those positive in the immunobead assay alone were the reverse (14 of 17 preoperatively; 5 of 15 postoperatively).

Thrombocytopenic controls. Platelets (15 patients) and plasma (20 patients) from 31 patients with nonimmune thrombocytopenia were studied. Negative results were obtained in every instance using the immunobead and the microtiter assays (data not shown).

Table 2. Antiplatelet Glycoprotein Autoantibodies: Group 1, Positive With Both Assays

				Anti-GP IIb/IIIa			Anti-GPIb		
				Immunobead			Immunobead		
Patient	Platelet Count/μL	SPLX	Resp	P-Assoc	Plasma	Well	P-Assoc	Plasma	Well
1	6,000	Yes	No	7.0*	9.4	Pos (640)	—	Neg	Neg
2	7,000	Yes	No	—	5.2	Pos (320)	—	Neg	Neg
3	11,000	Yes	No	84.5	28.5	Pos (80)	Neg	Neg	Neg
4	117,000	Yes	No	—	2.1	Pos (40)	—	Neg	Neg
5	1,000	Yes	No	—	—	Neg	—	3.3†	Pos (6,400)
6	3,000	Yes	No	—	Neg	Pos (40)	—	2.6	Pos (80)
7	7,000	Yes	No	—	Neg	Neg	17.2	2.0	Pos (20)
8	14,000	Yes	No	Neg	Neg	Neg	5.7	1.3	Pos (80)
9	15,000	No	—	Neg	Neg	Neg	43.1	19.4	Pos (40)
10	112,000	No	—	—	Neg	Neg	—	3.6	Pos (40)

Abbreviations: SPLX, splenectomy; Resp, response; Yes, normalization of platelet count; No, no response or relapse; Immunobead, results of platelet-associated (P-Assoc) or plasma immunobead assay expressed as a ratio of patient/control results (positive > 1.3); Well, results of microtiter-well assay expressed as negative (neg) or positive (pos) with titers of positive tests shown in parentheses.

*Plasma (0.1 mL) used to sensitize platelets.

†Platelets (0.18×10^8) assayed.

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Table 3. Antiplatelet Glycoprotein Autoantibodies: Group 2, Positive Immunobead Assay/Negative Microtiter-Well Assay

				Anti-GP IIb/IIIa			Anti-GPIb		
				Immunobead			Immunobead		
Patient	Platelet Count/μL	SPLX	Resp	P-Assoc	Plasma	Well	P-Assoc	Plasma	Well
11	2,000	Yes	Yes	16.7	2.3	Neg	—	Neg	Neg
12	3,000	Yes	No	—	2.2	Neg	—	Neg	Neg
13	4,000	Yes	No	6.3	2.0	Neg	Neg	Neg	Neg
14	4,000	Yes	No	5.0	Neg	Neg	Neg	Neg	Neg
15	9,000	Yes	No	—	4.5	Neg	—	Neg	Neg
16	13,000	Yes	No	—	2.5	Neg	—	Neg	Neg
17	14,000	Yes	No	—	1.5	Neg	—	Neg	Neg
18	19,000	No	—	—	1.4	Neg	—	Neg	Neg
19	21,000	Yes	No	—	3.8	Neg	—	Neg	Neg
20	23,000	Yes	Yes	—	1.7	Neg	—	Neg	Neg
21	23,000	No	—	3.8	Neg	Neg	Neg	Neg	Neg
22	29,000	Yes	No	48.0	Neg	Neg	Neg	Neg	Neg
23	34,000	Yes	Yes	—	1.6	Neg	—	Neg	Neg
24	40,000	Yes	No	30.1	5.5	Neg	Neg	Neg	Neg
25	40,000	Yes	No	17.0	2.6	Neg	Neg	Neg	Neg
26	50,000	Yes	No	16.1	3.9	Neg	Neg	Neg	Neg
27	50,000	No	—	10.0	2.5	Neg	—	Neg	Neg
28	53,000	Yes	No	2.2	Neg	Neg	Neg	Neg	Neg
29	106,000	Yes	Yes	16.2	1.4	Neg	—	Neg	Neg
30	145,000	Yes	Yes	—	3.1	Neg	—	Neg	Neg
31	7,000	Yes	No	Neg	Neg	Neg	21.8	3.4	Neg
32	19,000	Yes	Yes	Neg	Neg	Neg	2.4	Neg	Neg
33	33,000	No	—	Neg	Neg	Neg	15.4	1.6	Neg
34	39,000	No	—	—	Neg	Neg	—	2.0	Neg
35	44,000	Yes	No	Neg	Neg	Neg	18.2	Neg	Neg
36	69,000	No	—	Neg	Neg	Neg	4.9	1.8	Neg

DISCUSSION

In this report, we describe our experience with two clinically applicable methods for measuring antiplatelet autoantibodies in chronic ITP: the immunobead assay, which is capable of measuring both platelet-associated and plasma autoantibodies, and the microtiter assay, which is useful in detecting only plasma antibodies.

Platelet-associated autoantibodies were more likely to be positive. Positive results were noted in 75% of those tested (21 of 28 patients) and, in six of these, only platelet-associated autoantibodies were demonstrable. Plasma autoantibodies were noted less frequently (57.6% positive, 34 of 59 patients). Autoantibodies to GPIIb/IIIa occurred more commonly than did those to GPIb.

Autoantibody assays have distinct advantages over assays for platelet-associated IgG (PAIgG). First, they allow demonstration of autoantibodies against defined platelet proteins, confirming the autoimmune nature of the patient's disease. Conversely, the PAIgG assay measures IgG on platelets of unknown specificity. Second, autoantibody test results in patients with nonimmune thrombocytopenia have thus far been completely negative with these two assays, whereas PAIgG results are positive in many patients with a variety of diagnoses.[14,15] Although it seems likely, as suggested by the studies of Kelton et al,[15] that positive PAIgG results may reflect immune-mediated platelet destruction, an increase in PAIgG is not diagnostic of autoimmune thrombocytopenia. Although autoantibodies were not seen in the group of thrombocytopenic control patients reported here, when a large number of patients with collagen vascular disease or lymphoma are screened using these assays, positive results may likely be seen in some of these patient groups since they have other types of autoantibodies (eg, anti-RBC antibodies).

The difference in the results between the immunobead assay (36 of 59 positive) and the microtiter-well assay (14 of 59 positive) is of interest. Study of purified protein antigens shows that there are two types of epitopes: sequential and topographic.[13] Sequential epitopes involve amino-acid

Table 4. Antiplatelet Glycoprotein Autoantibodies: Group 3, Positive Microtiter-Well Assay/Negative Immunobead Assay

				Anti-GP IIb/IIIa			Anti-GPIb		
				Immunobead			Immunobead		
Patient	Platelet Count/μL	SPLX	Resp	P-Assoc	Plasma	Well	P-Assoc	Plasma	Well
37	9,000	Yes	No	—	Neg	Neg	—	Neg	Pos(40)
38	10,000	No	—	—	Neg	Pos(320)	—	Neg	Pos(80)
39	56,000	Yes	No	—	Neg	Neg	—	Neg	Pos(160)
40	85,000	Yes	Yes	—	Neg	Pos(640)	—	—	Neg

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Table 5. Antiplatelet Glycoprotein Autoantibodies: Group 4, Negative in Both Assays

Patient	Platelet Count/μL	SPLX	Resp	Anti-GP IIb/IIIa			Anti-GPIb		
				Immunobead			Immunobead		
				P-Assoc	Plasma	Well	P-Assoc	Plasma	Well
41	3,000	Yes	No	—	Neg	Neg	—	Neg	Neg
42	6,000	Yes	No	—	Neg	Neg	—	Neg	Neg
43	9,000	Yes	No	—	Neg	Neg	—	Neg	Neg
44	9,000	Yes	Yes	—	Neg	Neg	—	Neg	Neg
45	9,000	Yes	Yes	—	Neg	Neg	—	Neg	Neg
46	18,000	Yes	Yes	—	Neg	Neg	—	Neg	Neg
47	20,000	No	—	—	Neg	Neg	—	Neg	Neg
48	24,000	Yes	Yes	Neg	Neg	Neg	Neg	Neg	Neg
49	34,000	No	—	Neg	Neg	Neg	Neg	Neg	Neg
50	34,000	Yes	No	Neg	Neg	Neg	Neg	Neg	Neg
51	34,000	Yes	No	—	Neg	Neg	—	Neg	Neg
52	40,000	Yes	No	—	Neg	Neg	—	Neg	Neg
53	42,000	No	—	Neg	Neg	Neg	Neg	Neg	Neg
54	42,000	Yes	No	—	Neg	Neg	—	Neg	Neg
55	46,000	No	—	—	Neg	Neg	—	Neg	Neg
56	50,000	Yes	No	—	Neg	Neg	—	Neg	Neg
57	100,000	No	—	—	Neg	Neg	—	Neg	Neg
58	100,000	No	—	Neg	Neg	Neg	Neg	Neg	Neg
59	261,000	Yes	Yes	—	Neg	Neg	—	Neg	Neg

sequences of one section of the protein whereas topographic epitopes involve regions of the molecule remote in sequence but close in three-dimensional space due to the tertiary molecular structure. The most likely explanation for the greater percentage of positivity with the immunobead assay is that solubilization of the platelets prior to incubation with antibody, which is required for the microtiter assay, in some way disturbs the antigenic epitope whereas incubation of antibody and platelets prior to solubilization stabilizes it. We postulate that the microtiter assay measures sequential or stable topographic epitopes while the immunobead assay measures unstable topographic epitopes as well.

Alternatively, the immunoreactivity of the solubilized platelets may be affected by the freezing and thawing of the platelet lysate prior to coating the microtiter wells or binding of the GPs to monoclonal antibody on the microtiter well may alter the molecular configuration. Studies are in progress to evaluate these possibilities.

These assays are both adaptable to the measurement of other as yet unidentified autoantibodies when appropriate monoclonal antibodies become available. Because many patients with chronic ITP have no demonstrable autoantibody, autoantibodies against other platelet-associated antigens (eg, phospholipids, glycolipids, etc.) are probably present.

In summary, the present studies describe the use of two assays for measurement of autoantibodies to specific platelet proteins. Results show that most patients with chronic ITP have autoantibodies against either the platelet GPIIb/IIIa complex or against GPIb. Differences in the frequency of positive results in the two assays provide evidence for epitopes with varying degrees of stability on solubilization.

NOTE ADDED IN PROOF

In recent studies, we have substituted a single ¼ inch polystyrene bead (Pierce Chemical Co, Rockford, Ill) for the 100 mg of microbeads. Incubations with anti-human IgG and BSA are performed as described, except that we use 10 μg of HB-43 per bead; the beads are washed the same number of times by adding 10 ml of PBS-Tween/bead followed by aspiration of the fluid. After assay completion, the bead is transferred to a clean tube and the radioactivity determined. With this modification, the assay is simpler and the background radioactivity is much lower.

REFERENCES

1. McMillan R: Chronic idiopathic thrombocytopenic purpura. N Engl J Med 304:1135, 1981

2. Kelton JG, Gibbons S: Autoimmune platelet destruction: Idiopathic thrombocytopenic purpura. Semin Thromb Haemost 8:83, 1982

3. van Leeuwen EF, van der Ven JTH, Engelfriet CP, von dem Borne AEG: Specificity of autoantibodies in autoimmune thrombocytopenia. Blood 59:23, 1982

4. Woods VL, Oh EH, Mason D, McMillan R: Autoantibodies against the platelet glycoprotein IIb/IIIa complex in patients with chronic ITP. Blood 63:368, 1984

5. Woods VL, Kurata Y, Montgomery RR, Tani P, Mason D, Oh EH, McMillan R: Autoantibodies against platelet glycoprotein Ib in patients with chronic idiopathic thrombocytopenic purpura. Blood 64:156, 1984

6. Mason D, McMillan R: Platelet antigens in chronic idiopathic thrombocytopenic purpura. Br J Haematol 56:529, 1984

7. Beardsley DS, Spiegel JE, Jacobs MM, Handin RI, Lux SE: Platelet membrane glycoprotein IIIa contains target antigens that bind antiplatelet antibodies in immune thrombocytopenias. J Clin Invest 74:1701, 1984

8. Devine DV, Rosse WF: Identification of platelet proteins that bind alloantibodies and autoantibodies. Blood 64:1240, 1984

9. Varon D, Karpatkin S: A monoclonal antiplatelet antibody with decreased reactivity for autoimmune thrombocytopenic platelets. Proc Natl Acad Sci USA 80:6992, 1983

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10. Szatkowski NS, Kunicki TJ, Aster RH: Identification of glycoprotein Ib as a target for autoantibody in idiopathic (autoimmune) thrombocytopenic purpura. Blood 67:310, 1986

11. Nugent DJ, Berglund C, Bernstein ID: Isolation of platelet-specific human monoclonal antibodies using Epstein-Barr virus transformation and somatic cell hybridization. Proceedings of the INSERM Symposium on utilization of monoclonal antibodies for the understanding and detection of platelet activity. Elsevier, Amsterdam, 1986

12. Asano T, Furie BC, Furie B: Platelet binding properties of monoclonal lupus autoantibodies produced by human hybridomas. Blood 66:1254, 1985

13. Berzofsky JA: Intrinsic and extrinsic factors in protein antigenic structure. Science 229:932, 1985

14. Mueller-Eckhardt C, Mueller-Eckhardt G, Kayser W, Voss RM, Wegner J, Kuenzlen E: Platelet-associated IgG, platelet survival, and platelet sequestration in thrombocytopenic states. Br J Haematol 52:49, 1982

15. Kelton JG, Powers PJ, Carter CJ: A prospective study of the usefulness of the measurement of platelet-associated IgG for the diagnosis of idiopathic thrombocytopenic purpura. Blood 60:1050, 1982

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1987 70: 1040-1045

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