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Studies on the Specificity of Anti-Platelet Autoantibodies[1] (38424)

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We have described a procedure, the platelet factor 3 (PF-3) immunoinjury technique, for the detection of anti-platelet antibody (1, 2), which has been confirmed in several laboratories (3–6)[3]. Using this assay we have been able to demonstrate anti-platelet antibodies in 65% of patients with autoimmune thrombocytopenic purpura (ATP) and in 78% of patients with systemic lupus erythematosis (SLE). In addition, anti-platelet antibodies could be eluted from splenic tissues of some patients in whom serum antibody was not detectable (7). It was suggested that the term "idiopathic" thrombocytopenic purpura (ITP) be relegated to those patients without detectable anti-platelet antibody (8). The term ATP is used to refer to those patients in whom anti-platelet antibodies are detectable. Clinically and from the standpoint of platelet kinetics the two groups of patients are identical.

We have further shown that the anti-platelet auto-antibodies in ATP tend to be restricted to the γG3 subclass, while in SLE they are generally present in all four IgG subclasses (9). There was frequently a decrease in anti-platelet antibody titer following splenectomy (2, 7), and the spleen was shown to be a quantitatively important site for anti-platelet antibody synthesis (7). In ATP patients, antibody titer and severity of disease did not appear to be correlated. Results indicating the presence of anti-platelet antibodies in ATP have recently been published by a number of workers using a variety of different techniques (3–6, 10–14).

In our earlier studies (1) it was apparent that, when PF-3 assays on the same serum were repeated using different samples of platelet rich plasma, different values for the antibody titer were occasionally obtained. In the work reported here we have attempted to answer the following questions: (a) Do anti-platelet antibodies from different patients have the same specificity? (b) Do platelets from different subjects differ in their interaction with anti-platelet antibodies? (c) Does a patient's antibody react more "intensely" with his own platelets than with platelets from unrelated donors?

Materials and Methods. Measurement of anti-platelet antibody. Anti-platelet antibodies were assayed by the PF-3 method (2) using globulin fractions of serum prepared by precipitation with 50% cold, saturated ammonium sulfate. Anti-platelet antibodies are detected by their ability to cause platelet damage as indicated by a shortening of the fibrin clotting time in the PF-3 assay. The data are presented either as the percent change in fibrin clotting time when comparing the patient's globulin with a simultaneously run control of normal human pooled globulin or as the maximum dilution of the patient's globulin producing a significant shortening of the fibrin clotting time. The fibrin clotting time obtained for repeated PF-3 assays using control globulin was 90 ± 9 sec (average ± 2SD). Thus, a shortening of the fibrin clotting time by greater than 9 sec (or > 10%) may be regarded as significant. It should be noted that all measurements reported were repeated two or three times and each time done in duplicate.

Serum samples. Sera were obtained from patients attending the Bellevue Hospital SLE or Hematology Clinics or from other hospitals. The

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patients had histories and laboratory findings consistent with a diagnosis of ATP or SLE, or had unexplained thrombocytopenia. We are concerned here with the immunochemical specificity of anti-platelet antibodies and not with the particular disease states in which they appear. When several samples from an individual patient were studied, the sera were stored at −20° and all assays were carried out on the same day. A pooled normal control was run with each platelet-rich plasma preparation. When individual samples were assayed against several different platelet-rich plasmas, the same normal control globulin was used for all assays. A series of assays was also performed in which ATP sera were assayed both against the patient's own platelets and against platelets from an unrelated donor. In each case, the patient's platelets were obtained during remission (often postsplenectomy) when platelet counts had returned to normal.

Circulating immunoglobulin levels. Serum levels of IgG, IgA, and IgM were determined on globulin fractions made up to the original serum volume with PBS using the radial immunodiffusion technique of Mancini *et al.* (15). Comparative measurements on serum and globulin fractions from a group of five subjects gave similar results.

Experimental results. Effect of varying the source of the platelet-rich plasma used in the PF-3 test. In Table I are summarized the results of assaying, by the PF-3 test, a series of globulins from individual patients using platelets obtained from a panel of donors. In general, similar results are obtained when antibody activity is assayed against platelets from different donors. However, it is clear that in some cases definite differences in the PF-3 test are observed when different platelets are used. For example, globulin from patient and, which causes a 51% shortening of the fibrin clotting time in the PF-3 test with platelet-rich plasma of NS causes no shortening with platelets from AW or FB. Platelets from three other donors gave clearly positive reactions with this globulin but the degree of shortening of the fibrin clotting time tended to be less than with platelets from NS.

Examination of the data in Table I suggests that platelets from certain donors tend to be more sensitive indicators of antibody activity. That is, platelets from different donors appear to differ in susceptibility to antibody-mediated release of PF-3. For example, platelets from NS, LS, and RS appear to be particularly sensitive indicators of anti-platelet activity while platelets from FB and SK tend to be generally less sensitive.

It should be noted that, in addition to variation in the sensitivity of platelets from different donors to antibody-mediated PF-3 release, different antibody samples appear to differ in their specificity. That is, individual sera vary in the extent to which they cause PF-3 release in a manner which suggests that the autoantibodies actually differ in regard to their detailed specificity. For example, Kri causes markedly greater, and Bec slightly greater, release of PF-3 from LS as compared with MK platelets. However, the other five samples tested using these two platelet donors all caused equivalent release or slightly greater release from MK. Similarly, globulin from patient Koo released more PF-3 with platelets from AW as compared with DS while globulin from Mot showed the reverse specificity.

PF-3 test using platelets from ATP patients. Globulin fractions of sera from five ATP patients were tested for anti-platelet antibodies by the PF-3 assay using their own platelet-rich plasma and platelet-rich plasma from an unrelated donor. Platelets from ATP patients were obtained postsplenectomy or during remission when the patient's platelet counts had returned to normal. The data are presented in Table II. In three of the patients studied (Stl, Ruo, and Ter) higher anti-platelet antibody titers were obtained by the PF-3 test when autologous as compared with homologous platelets were used. In two of the patients (Coe and Bad) identical titers were seen with homologous and autologous platelets. These studies were performed on coded samples by a "double-blind" technique.

Circulating immunoglobulin levels in patients with ATP vs ITP. Preliminary studies on the immunoglobulin levels of patients with ATP and ITP were carried out. Both groups of patients generally had normal levels of IgG, IgM, and IgA. However, out of 32 ITP patients seven had IgG levels above 1500 mg/100 ml, seven had IgA levels above 400 mg/100 ml, and eight had IgM levels above 180 mg/100 ml. Only one of 17 ATP patients studied had an elevated immunoglobulin level, and this was for IgM. It is of interest that at least five of the ITP patients with elevated IgG levels, six of the ITP patients with elevated IgM levels, and three of the ITP patients

TABLE I. Anti-Platelet Antibody Results Using Platelets from Different Donors.[a]

Globulin fraction	Age, sex, disease[b]	Percent of control PF-3 test with a panel of platelet-rich plasmas									
		NS	MF	MK	LS	SK	AW	FB	IK	DS	RS
And	12 F Hodgk*	51	—	73	79	83	120	107	—	—	—
Car	4 F ATP	66	—	77	86	81	84	—	—	—	—
Kri	27 F ATP	—	52	86	49	73	70	83	—	—	—
Coh	79 M Pancyt	—	87	106	99	89	—	98	—	—	—
Pol	50 F ATP	—	—	71	75	68	70	90	—	—	—
Bec	58 M CML*	—	—	89	77	77	88	120	—	—	—
Mar	12 M Inf Mono	—	—	106	112	108	93	—	—	—	—
Gra	32 M SLE	—	—	—	—	—	—	—	90	—	65
Wil	57 F SLE	—	—	—	—	—	—	—	90	—	54
C6	64 M RTR*	—	—	—	—	85	106	—	—	—	—
C7	67 M RTR*	—	—	—	—	83	74	—	—	—	—
C8	44 F RTR*	—	—	—	—	82	74	—	—	—	—
Koo	57 M LSA	—	—	—	—	—	38	—	—	77	—
Vel	9 M ITP	—	—	—	—	—	114	—	—	102	—
Mot	38 F ATP	—	—	—	—	—	84	—	—	74	—
Lud	77 F ATP*	—	—	—	—	—	123	—	—	83	—
Bro	34 F ATP	—	—	—	73	—	75	—	—	—	—

[a] PF-3 assays with globulin fractions of sera from a series of patients were carried out using platelet-rich plasma from a panel of donors. The data are presented as percent of control clotting time. The control clotting time was obtained from a pooled globulin fraction. The data are averages of two or three independent determinations each done in duplicate. Repeat determinations in all cases were qualitatively comparable with respect to the direction of the differences observed.

[b] Abbreviations: ITP, idiopathic thrombocytopenic purpura; ATP, autoimmune thrombocytopenic purpura; SLE, systemic lupus erythematosis; CML, chronic myelocytic leukemia; RTR, renal transplant rejection; Info Mono, infectious mononucleosis; * indicates patients who had history of multiple platelet or blood transfusions. Other patients do not have history of transfusion.

with elevated IgA levels appeared to have particularly severe disease clinically and to be refractory to therapy with steroids or splenectomy.

Discussion. We have shown that platelets obtained from different donors differ with regard to the extent to which they release PF-3 upon interaction with anti-platelet antibody. Occasionally, a situation may occur where antibody can be detected with particularly sensitive platelets but not with less sensitive platelets. This finding should be borne in mind when assays for anti-platelet antibodies are carried out by procedures such as the PF-3 test which involve the demonstration of platelet damage. The basis for this platelet heterogeneity is not known. It would seem more likely that this difference in behavior of different platelets is due to some physiologic variation in the platelets rather than to antigenic variation.

In addition to the variations in sensitivity of different platelet preparations, individual anti-platelet antisera reacted to different extents with platelets from different donors. Similar results have recently been reported by Hirschman and Gralnick (6), who employed a simplified modification of our PF-3 test. These observations clearly suggest that anti-platelet autoantibodies made by different patients differ in detailed specificity.

It was also shown that the anti-platelet antibodies of some, but not all, ATP patients release PF-3 from their own platelets at higher globulin dilution than with platelets of an unrelated donor. Three possible explanations for this observation may be considered: (a) Platelets of patients who develop ATP are intrinsically more susceptible to damage by anti-platelet antibody. This might help explain why patients with SLE often have high titers of anti-platelet antibody but insignificant thrombocytopenia. (b) Platelets from patients with ATP are already partially coated with antibody as a consequence of their *in vivo* exposure and are thus more sensitive to antibody *in vitro*. However, this would not explain why some ATP patients show this preferential reactivity with their own platelets and some

TABLE II. PF-3 Results Testing Globulin Fraction of ATP Patients with Their Own Platelet-Rich Plasma and with Control Platelet-Rich Plasma.[a]

Patient	Sample[b]	Anti-Platelet Antibody Titers	
		Control Platelets	Patient's Platelets
Stl	Pre-1	0	0
	2	4	8
	3	16	36
	4	0	4
	5	0	0
	6	0	4
Coe	Pre-1	4	4
	2	4	4
	3	4	4
	4	4	4
	5	0	0
Ruo	Pre-1	8	40
	2	0	8
Bad	1	36	40
	2	32	32
	3	12	16
	4	4	8
Ter	Pre-1	20	80
	2	8	24

[a] Data are presented as the reciprocal of the highest dilution of the patient's globulin causing significant shortening of the fibrin clotting time. Measurements were repeated two and in some cases three times, each time in duplicate to confirm the results.

[b] Pre-one relates to samples obtained presplenectomy. Other samples were obtained at various intervals postsplenectomy.

do not. (c) It is possible that these findings reflect some individual specificity of the anti-platelet autoantibodies. That is, while these antibodies mainly behave as "panantibodies," reacting with all human platelets, they may, in some patients, show a degree of individual specificity and react better with the patient's own platelets. Indeed, in Harrington's original *in vivo* infusion studies, only 65% of patients with ITP had a factor in their plasma which was capable of lowering the platelet count in volunteer recipients (16). This observation is consistent with the data suggesting variation in specificity of anti-platelet antibodies from different patients. It is not possible at present to choose between these three explanations.

Preliminary observations suggested that there was a tendency towards greater severity of disease and refractoriness to treatment (steroids and/or splenectomy) in those ITP patients with elevated IgG or IgM levels. We have no explanation for these observations but it is conceivable that the ITP patients with elevated immunoglobulin levels actually have a different disease than the conventional ITP or ATP.

Summary. Platelets from different donors varied in their sensitivity to anti-platelet antibody, as determined by the platelet factor 3 (PF-3) immunoinjury technique. Anti-platelet autoantibodies from different patients also differed in their detailed specificity. Anti-platelet autoantibodies of some patients with autoimmune thrombocytopenic purpura exhibited higher titers in the PF-3 test when the patient's own platelets were used as compared to platelets from an unrelated donor.

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