Platelet Migration Inhibition: A New Method for Detection of Platelet Antibodies

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A test for platelet antibodies based on the inhibition of migration of platelets from a capillary tube is described. The technique is simple and is capable of detecting antibodies directed against HL-A antigens and the platelet-specific antigen, PlA1. Inhibitory activity was detected in sera from two of seven patients with idiopathic thrombocytopenic purpura. The platelet migration inhibition test was as sensitive as platelet lysis and more sensitive than complement fixation, aggregometry, and platelet factor-3 release for detection of the antibodies studied.

RECENT REPORTS HAVE SHOWN that platelets are capable of migrating from capillary tubes in vitro.1,2 This migration is not the result of diffusion or Brownian movement but may be due to platelet motility.3,4 By analogy with macrophage migration,5 it seemed possible that migration of platelets could be inhibited immunologically. This report describes a method of platelet migration inhibition (PMI) by antibody. Selected sera containing antibodies against HL-A and PlA1 antigens of platelets and serum from a patient with immune idiopathic thrombocytopenic purpura each were found to be capable of inhibiting platelet migration by immunologically specific reactions. The sensitivity of the PMI test for detection of platelet antibodies was comparable to that of other methods of platelet antibody detection.

MATERIALS AND METHODS

Freshly drawn blood anticoagulated with 0.20 parts of 20% w/v sodium citrate per 10 parts of blood was centrifuged at 450 g at room temperature for 9 min in plastic tubes. The upper two-thirds of the layer of platelet-rich plasma (PRP) was removed and placed into a second tube. A portion of the PRP was centrifuged at 1200 g for 8 min to produce platelet-poor plasma (PPP), which was placed in a separate tube. In a plastic tuberculin syringe or tube, 0.3 ml of PRP (1-2 x 10^8 platelets) was mixed with 0.2 ml serum to be tested, which had previously been heated at...
56°C for 30 min. Test serum was diluted in pooled normal serum for titration studies. After incubation at room temperature for 60 min, the mixture was placed into a glass capillary tube made by cutting off the tip of a Pasteur pipette, heat-sealing it at the capillary end, and silic-}

conizing it. After centrifugation at 1200 g for 3.5 min, the capillary tube was cut approximately 1 mm above the surface of the platelet button. The segment containing the button was placed in a clear plastic circular migration chamber with a diameter of 2 cm (Mini-Lab, Quebec, Canada) and anchored with a small amount of silicone grease. The migration chamber was sealed with a clear cover glass and paraffin wax and filled with 0.5 ml of medium consisting of 0.3 ml of Eagle’s Minimal Essential Medium (GIBCO, Grand Island, N.Y.), 0.2 ml of autologous PPP, 100 units of penicillin, and 10 µg of streptomycin. All tests were done in duplicate or triplicate. After incubation at room temperature for 12–24 hr, the area of platelet migration was magnified 45-fold with a microprojector (Bausch and Lomb). The projected image was drawn on a sheet of paper, and the area of migration was measured with a polar planimeter. One unit of migration area was equivalent to 83 sq cm.

The sensitivity of the PMI test for platelet antibody detection was compared to that of several other immunologic techniques. The platelet lysis test in which 31Cr is released from platelets in a complement-dependent reaction was performed as described by Aster and Enright. The sensitivity of the technique has been shown to be equal to, or greater than, that of conventional complement fixation testing. Measurement of antibody by platelet aggregometry was carried out as described by Deykin and Hellerstein. Platelet factor-3 release tests for platelet antibodies were performed as described by Karpatkin et al. and by Hirschman and co-workers.

Antibodies utilized in comparative studies were obtained from multiparous females (anti-HL-A), patients with posttransfusion purpura (anti-Pl(A)) and patients with chronic idiopathic thrombocytopenic purpura (ITP). Methods by which specificities of the former antibodies were confirmed, and criteria for the diagnosis of ITP have been previously described.

RESULTS

Figure 1 shows a typical example of the 24-hr migration patterns of platelets incubated at room temperature with varying dilutions of lymphocytotoxic HL-A antiserum Eis (anti-HL-A2, W28) and normal serum. Dilutions of this antiserum up to 1:16 gave complete inhibition of migration. With a dilution of 1:32, the area of migration was 20% of that observed with normal serum. Higher dilutions caused little or no inhibition. Inhibition of platelet migration was not observed when platelets were incubated with serum from 40 normal subjects.

Fig. 1. Migration patterns of HL-A2 positive platelets incubated with (a) anti-HL-A2 serum diluted to 1:16, (b) with anti-HL-A2 serum diluted to 1:32, and (c) normal serum. Inhibition of platelet migration by antiserum is shown by marked decreases in migration areas as compared to controls.
Fig. 2. Migration areas of HL-A2 (or W28) positive platelets incubated with serum Eis (anti-HL-A2, W28) in different dilutions. Control serum was pooled normal human serum. Considerable inhibition of migration was observed with serum Eis in dilutions as high as 1:32.

In preliminary studies, the anticoagulant used was found to influence the migration patterns of platelets. The greatest area of migration was generally observed with platelets prepared from blood anticoagulated with ethylene diamine tetraacetate (EDTA) 0.16% w/v. Somewhat less migration was observed with citrate as anticoagulant and considerably smaller areas of migration were seen with platelets obtained from heparinized blood. On the other hand, the inhibitory role of antiplatelet antibody on platelet migration was most pronounced with platelets extracted from citrated blood. Citrate was therefore used as the anticoagulant for all subsequent studies.

Initial studies of the effect of temperature on migration of platelets demonstrated that the areas of migration were consistently greater after incubation at 25°C than at 37°C. Therefore, in all studies the platelets were allowed to migrate at room temperature.

The inhibitory effect of various dilutions of serum Eis (anti-HL-A2, W28) on the migration of platelets from normal donors positive for antigens HL-A2 and W28 was studied. Complete inhibition of platelet migration occurred with dilutions as high as 1:16 (Fig. 2). While the titration curves were quite similar, variations were observed in the migration areas of platelets obtained from five donors positive for HL-A2 or W28. The variations in migration were unrelated to the number of platelets present in the capillary tubes (range: 1–2 × 10^8 platelets) or to ABO incompatibility between platelet donor and serum donor. Inhibition of platelet migration by serum Eis was specific for HL-A2 or W28 positive platelets, since when platelets negative for these antigens were used, no inhibitory effect of this serum on platelet migration could be demonstrated (Fig. 3). The failure of serum Eis to inhibit migration of HL-A2-negative platelets was not due to a general unresponsiveness of such platelets to specific antisera, since incubation of the same platelets with antisera against other HL-A antigens present in the donor (HL-A1 or HL-A5) cause PMI (Table 1). Thus, the inhibition of platelet migration by anti-HL-A antibody is an immunologically specific phenomenon.

The PMI test and the platelet lysis test were comparable with respect to
sensitivity for antibody detection (Fig. 4). The titration curves observed with serum Eis in the platelet lysis and PMI tests were similar, and titers of 1:16 were observed in both tests, using platelets from two different HL-A2 positive donors. Chromium was not released from platelets of HL-A2 negative donors. The platelet lysis and PMI tests also gave similar titration scores using an antiserum (Hin) reacting with the platelet-specific antigen, PIA1 (Fig. 5). Comparative studies showed that the platelet aggregometry7 and PF-3 release tests,8,9
were less sensitive than either PMI or platelet lysis for detection of serum Eis (anti-HL-A2), Hin (anti-PI\(^A\)) and Dav (auto-antibody of ITP) (Table 2).

Sera from patients with idiopathic (autoimmune) thrombocytopenic purpura (ITP) were also capable of inhibiting platelet migration. An example of the migration inhibitory activity of serum from a patient with ITP is shown in Fig. 6, together with the results of the platelet lysis test using platelets from the same donor. Although low titers were observed for this serum, there was general agreement between the results of the platelet lysis and PMI tests. Of a total of seven sera from patients with ITP tested, two gave positive results in both tests. None of the sera from the patients with ITP reacted in the lymphocytotoxicity test against a panel of 50 lymphocyte donors. Thus, it is unlikely that the antiplatelet activity detected by PMI was due to anti-HL-A antibodies stimulated by previous transfusion or pregnancy.
DISCUSSION

The PMI test is relatively simple and can be adapted to almost any laboratory. With the antibodies studied, it proved considerably more sensitive than aggregometry, and platelet factor-3 release, another relative simple technique, and at least as sensitive as platelet lysis.* Although we have not yet shown that the PMI-inducing activity of serum resides in the gamma globulin fraction, its antibody nature appears certain from the immunologic specificity manifested by serum Eis, which only inhibited migration of platelets positive for HL-A2 or W28. Moreover, serum from 40 normal subjects had no inhibitory properties. These observations suggest that the PMI test is a useful addition to the battery of techniques capable of demonstrating platelet antibodies.

Of interest, but yet unexplained, is the variability of the area of migration of platelets from different donors or from the same donor obtained on different occasions. Variations in migration area make the interpretation of the results with weaker sera somewhat more difficult.

Lowenhaupt, Miller, and Glueck,3 who also studied platelet migration, have suggested that it is a physiologic phenomenon and not merely the result of diffusion or Brownian movement. They demonstrated that human platelet migration could be directed chemotactically by collagen,3 and that cytochalasin B, a known inhibitor of motility of many types of cells, caused significant inhibition of migration.4 Valone, Goetzl, and Austin3 have adapted the Boyden micropore filter to measure the migration of human platelets in vitro, and showed that migration could be inhibited by bivalent cations and by iodoacetamide, a suppressor of glycolysis. We have observed that platelet migration from capillary tubes can be inhibited by epinephrine in concentrations capable of inducing platelet aggregation. On the other hand, adenine diphosphate (10–100 μM), which also causes platelet aggregation, fails to induce significant inhibition. When EDTA, rather than citrate, was used as anticoagulant, the ability of epinephrine to induce platelet aggregation was greatly diminished, and inhibition of migration could be observed only with very high doses of epinephrine. Platelets obtained from blood anticoagulated with EDTA exhibited an extremely high rate of migration from capillary tubes, which was almost complete after 30 min at room temperature, whereas at least 12 hr were required for migration of platelets from citrated blood. Migration of platelets obtained from EDTA blood may, therefore, not represent a true physiologic phenomenon, but a passive one related to altered electrostatic charge on the platelet surface. On the other hand, migration of citrated platelets may provide a new method for assessment of platelet function.

REFERENCES

3. Lowenhaupt RW, Miller MA, Glueck HJ:

*Based on the use of untreated platelets. Treatment of normal platelets with the enzyme bromelain10 or use of platelets from patients with paroxysmal nocturnal hemoglobinuria* increases the sensitivity of the platelet lysis test four- to eightfold.


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