Hyperaggregation effect in idiopathic thrombocytopenia and multiple transfused patients

NORMAN WAGNER AND THOMAS J. DEGNAN

From the Laboratories, North Shore Hospital, Manhasset, New York, and the Department of Medicine, Cornell University Medical College, New York, New York, USA

SYNOPSIS A new technique for detecting platelet antibodies was investigated. The serum from patients having platelet antibodies was added to normal group O compatible pooled platelet-rich plasma. Platelet aggregation induced by adrenaline and adenosine diphosphate (ADP) was significantly increased as compared to the identical platelet-rich plasma without serum. The platelet-rich plasma with serum added did not aggregate spontaneously. When an alpha-adrenergic blocking agent (tolazoline) was added, the primary wave of aggregation induced by adrenaline was equally reversed with platelet-rich plasma, with or without serum.

Although platelet antibodies by themselves did not agglutinate platelets in our test system, it is probable that platelet antibodies interact with platelets and potentiate their ability to aggregate. It is suggested that the platelet aggregometer is a useful tool for the detection of platelet antibodies in vitro.

The detection of antibodies in patients with idiopathic thrombocytopenia and patients receiving multiple platelet transfusions has included a variety of different tests. These tests employ direct visual assays such as platelet lysis (Zucker, Ley, Borrelli, Mayer, and Firmat, 1959), platelet agglutination (Harrington, Sprague, Minnich, Moore, Aulvin, and Dubach, 1953), and inhibition of clot retraction (Ackroyd, 1949).

A more sensitive and reproducible test for the detection of platelet antibodies is platelet factor 3 activation (Karpatkin and Siskind, 1969; Horowitz, Rappaport, Young, and Fujimoto, 1965; Clancy, Jenkins, and Firkin, 1972).

The aim of this investigation is to describe a new technique for determining platelet antibodies. The assay employs a platelet aggregometer which records optical changes in platelet-rich plasma when sensitized platelets are induced to aggregate. The method is simple, qualitative, and reproducible.

Methods

Patients
Twenty-five control subjects were chosen at random. Thirteen abnormal patients were studied, seven of whom were diagnosed as having idiopathic thrombocytopenia. These patients had bone marrows with increased numbers of normal megakaryocytes but no infiltrative lesions or other associated disease states. The remaining patients had leukaemia and received multiple platelet transfusions. They also had received chemotherapeutic agents but no specific immunosuppressive drugs.

Collection of Platelet-rich Plasma
The blood from five healthy group O donors was drawn into tubes containing 3.8% sodium citrate (1 part citrate: 9 parts blood), and centrifuged at 250 × g for 15 minutes. The platelet-rich plasma from the five donors was pooled and placed in a plastic test tube which was allowed to stand at room temperature. An aliquot of pooled platelet-rich plasma was centrifuged at 1800 × g for 15 minutes to obtain platelet-poor plasma. The plasma obtained from these donors was used for the platelet function studies.

Collection of Serum
Blood from the 25 normal control subjects and 13 patients was drawn and then incubated at 37°C for 30 minutes and allowed to clot. The serum samples were removed after centrifugation and were incu-bated at 56°C for 30 minutes in order to inactivate any residual thrombin.

Received for publication 26 June 1973.
PLATELET FACTOR 3 ACTIVITY
This test, which demonstrates immune reactions involving platelets, has already been described (Clancy et al, 1972).

AGGREGATION STUDIES
Platelet aggregation was measured turbidimetrically with a chrono-log aggregometer according to the method of Born (1962). A Bausch and Lomb VOM 5 recorder was used to record the change in light transmission.

Twenty microlitres of heat inactivated serum was added to 0.5 ml of platelet-rich and 0.5 ml of platelet-poor plasma. The platelet-rich serum was stirred at 1200 rpm at 37°C. The aggregometer was adjusted such that the difference in transmitted light between platelet-rich and platelet-poor serum gave a pen deflection of 80% of full scale. This deflection was taken as 100% aggregation. Controls substituting normal heat-inactivated serum were run parallel with patients’ serum. Platelet-rich serum was incubated for five minutes at 37°C before the induction of aggregation. Platelet aggregation was induced by adrenaline, 10 µg/ml and 0.1 µg/ml, final concentration (Upjohn Co.), and by ADP, 2 µM and 0.01 µM, final concentration (Sigma Chemical Co.). Adrenaline (10 µg/ml, final concentration)-induced aggregation was also assayed in combination with tolazoline (25 µg/ml, final concentration). In this test, tolazoline was administered after the onset of the primary wave of aggregation induced by adrenaline.

Results
Platelet antibodies were demonstrated in 12 of the 13 patients with the platelet factor 3 activity test (table). Aggregation studies showed a potentiation of aggregation of platelet-rich serum in 12 of 13 patients with adrenaline and ADP-induced aggregation (0.1 µg/ml and 0.01 µM respectively). No significant difference in percentage aggregation between the normals and the patients was found using higher concentrations of adrenaline (10 µg/ml and AD (2 µM). The average increase in aggregation compared with that in normal controls was 6.1 times greater with adrenaline (0.1 µg/ml) and 6.5 times greater with ADP (0.01 µM).

Tolazoline (25 µg/ml) completely reversed adrenaline-induced aggregation of platelet-rich serum.

Discussion
The use of the platelet factor activity test has been reported to be a reliable method for demonstrating immune/injury to platelets (Karpatkin and Siskind, 1969; Horowitz et al, 1965).

In our study we used the modified method of Clancey et al (1972) since this method gave us more reproducible results. As expected, we found that platelet antibodies were present in patients with idiopathic thrombocytopenia and in leukaemic patients who received multiple transfusions. We have also shown that platelet antibodies can be assayed by using a platelet aggregation technique. The presence of platelet antibody determined by platelet factor 3 activity was confirmed in every case by aggregation assays. In one case, a patient with idiopathic thrombocytopenia did not demonstrate a platelet antibody by either method. We are aware that serum derived from normal patients would contain less prothrombin, more platelet factor 3 and ADP than idiopathic thrombocytopenia serum. However, since we are adding small aliquots of serum to pooled platelet-rich plasma rich in these factors, these differences in serum cannot account for the large differences in platelet factor 3 and aggregation between normals and patients with idiopathic thrombocytopenia. We investigated the effects of an alpha-adrenergic blocking agent (tolazoline) in order to elucidate the mechanism of potentiated aggregation. We have found that tolazoline will reverse the primary wave of platelet aggregation induced by adrenaline but will not reverse the secondary wave. We added tolazoline after the primary wave of aggregation in order to investigate the possibility that platelet antibody might be able to cause an irreversible aggregation, once the platelets were initially induced to aggregate. Prompt reversal of adrenaline-induced aggregation occurred with platelet-rich serum, suggesting that enhanced aggregation rather than irreversible agglutination occurred.
Hyperaggregation effect in idiopathic thrombocytopenia and multiple transfused patients

The study by Deykin and Hellerstein (1972) showed that drug-dependent immune antisera cause platelet lysis. Perhaps this mechanism was involved in our test system, where the sensitized normal platelets upon lysis released their intracellular ADP which in turn potentiated aggregation induced by adrenaline and ADP. In the same study by Deykin and Hellerstein, the serum from seven patients with idiopathic thrombocytopenia failed to induce direct aggregation of normal platelet-rich plasma.

Although these workers did not study the effects of potentiation by aggregating agents, they did show that serum from two patients who were multi-transfused with platelets could induce direct aggregation with normal platelet-rich plasma. Although we could not demonstrate this effect, it is possible that the immune serum from the patients in our study were of low titre and therefore failed to show any direct induced aggregation.

The use of an aggregation assay system for the detection of platelet antibodies is a simple and sensitive technique. The assay is sensitive to abnormalities of patients with idiopathic thrombocytopenia and patients several times transfused with platelets.

References