The Origin of ABH Antigens on Human Platelets

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ABH antigens are present on platelets from individuals of the corresponding red cell phenotype, but the extent to which these antigens are intrinsic or adsorbed remains undefined. To evaluate platelets for intrinsic H substance, an IgM mouse monoclonal antibody against type 2H chain (the intrinsic H structure found on erythrocytes) was labeled with 125I and incubated with platelets from donors of different ABO type. The antibody showed dose-response saturation curves, and binding to platelets paralleled that of the red cell ABO type, with 0 > B > A1 > A1B > O, cells, giving a single factor variance F of 190 (P < .0005). Passive adsorption of A antigens by platelets has been previously reported. To verify this phenomenon for A and B antigens and to quantitate the elution of A and B antigens from platelets, the following assay system was used. Platelets from group A, B, and donor donors were incubated in plasma from group 0 donors, and platelets from group 0 donors were incubated in plasma from different ABO.

The circulating human platelet readily acquires both ABH and HLA antigens by passive adsorption from the surrounding plasma. The soluble ABH antigens involved in this phenomenon presumably reside on glycolipid molecules possessing the type 1H chain, while ABO antigens intrinsic to red cell membranes are composed of type 2H chains. These two precursor molecules of the A and B antigens differ in the linkage of the subterminal galactose to N-acetyl-galactosamine, with type 1 chain showing β, 1–3 linkage and type 2 chain involving β, 1–4 linkage. Although platelets are known to possess the ABH antigens corresponding to the ABO red cell type of the individual, the extent to which these platelet antigens are intrinsic (type 2H chain) and adsorbed (type 1H chain) has not been delineated.

We have used radioimmunoassays, sensitive over a wide range of antigen and antibody concentrations, to examine the human platelet for intrinsic and adsorbed ABH antigens. Evaluation of intrinsic antigens was performed using a direct assay with radiolabeled mouse monoclonal anti-2H antibody.

The adsorption of A antigen onto group O platelets and the subsequent elution of adsorbed antigen over a 60-minute time course has been studied by Kelton et al., who used a mixed erythrocyte-platelet agglutination test. This assay is subject to a number of errors because of nonspecific cell clumping and the difficulty of visual interpretation. In addition to confirming the rate of A antigen adsorption onto group O platelets with our assay, we wished to verify that the same process occurs with B antigen. Furthermore, we have studied the elution of both A and B antigen from these "coated" platelets and from genetic group A and B platelets over a 24-hour time period.

A two-stage assay with radiolabeled mouse monoclonal anti-human IgG was used to evaluate binding of anti-A and anti-B to platelets incubated in plasma from various ABO, Lewis, and presumed secretor-type donors. The presence and variability of intrinsic (2H) and extrinsic (1H) antigens on platelets may affect the recovery and survival of transfused platelets when donor-recipient mismatches involve the ABO system.

MATERIALS AND METHODS

Collection of Cells and Serums

Whole blood, cells, and serums were collected from staff members in the Duke University Medical Center Laboratories. Fresh and previously frozen human serums were used. The human group O serums containing IgG anti-A or anti-B were supplied by the Carolinas Region American Red Cross Blood Service. An IgG fraction was prepared from each serum by precipitation with caprylic acid and ammonium sulfate. Antisera were screened before use by the microlymphocytotoxicity technique to verify the absence of HLA antibodies and by standard blood banking techniques to exclude irregular red cell antibodies. Collection and preparation of platelets were as previously described. Platelets

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from a donor of the O, blood group were supplied by the American Red Cross (Buffalo, NY) in citrate phosphate dextrose-adrenaline (CPD-A1) and tested within 24 hours of collection. CPD-anticoagulation and storage at 20°C is considered optimal for platelet antibody assays when platelets cannot be tested immediately. Group O one-day-old platelets collected in the same anticoagulant gave results not significantly different from those of group O platelets collected in EDTA.

**Purification and Radiolabeling of Monoclonal Antibodies**

Mouse monoclonal anti-human IgG (Fc) (Bethesda Research Laboratories Inc, Gaithersburg, Md) was purified from ascites fluid and radiolabeled as previously described. Mouse monoclonal IgM anti-human type 2H erythrocyte antigen was purified from ascites fluid as follows: 2 ml of ascites fluid was precipitated in saturated ammonium sulfate, dissolved in distilled water, and dialyzed at 4°C for 60 hours in TRIS-HCl (all at pH 8.0). The dialysate was applied to a diethyl aminoethyl (DEAE) cellulose column equilibrated in the same buffer. The protein was then eluted in 0.2 mol/l KCl in 0.01 mol/l TRIS HCl (pH 8.0) and concentrated before chromatography on Sephadex G-200 with a cellulose column eluted in the same buffer. The protein was then eluted in 0.2 mol/l KCl in 0.01 mol/l TRIS HCl (pH 8.0) and concentrated before chromatography on Sephadex G-200 with TRIS- NaCl buffer (0.1 mol/l TRIS-HCl, 0.15 mol/l NaCl, and 0.005 mol/l sodium azide). Fractions were pooled and purity was determined by polyacrylamide gel electrophoresis (PAGE). The purified antibody contained minor (less than 5%) contamination with other proteins by sodium dodecyl sulfate (SDS)-PAGE. The antibody was preserved in frozen fractions that were thawed for use and radiolabeled as for the monoclonal anti-human IgG.

**Radioimmune Assay**

To measure the quantity of type 2H antigen present on platelets, a direct (one-stage) assay system was used to compare the binding of monoclonal anti-type 2H antibody to platelets from different ABO blood group donors. Equal volumes (100 μl) of platelets and 125I-labeled anti-type 2H antibody were added together for 30 minutes at 22°C. Anti-type 2H antibody, labeled with platelets paralleled that predicted for red cells of the same phenotype (Fig. 1). Single-factor analysis of variance gave an F value of 190 (P < .0005), while analysis by Student-Newman-Keulls test also demonstrated statistically significant differences in type 2H antigen strength on platelets of different ABO types. The strength of the type 2H antigen on platelets corresponds to the erythrocyte pattern of O > B > A1 > A1B > O, phenotypes and is unaffected by the Lewis and secretor type of the platelet donor. Dose-response curves with anti-type 2H against O, A1, and B platelets demonstrate that the type 2H structure is a saturable receptor on platelets (Fig 2).

Group O platelets incubated with A or B plasma acquire A or B antigen reactivity, which increases rapidly during the first hour and then more slowly.
from one to 24 hours (Fig 3). Group O platelets incubated in O plasma or buffer show no A or B activity. Group O platelets incubated with A or B plasma for five hours, washed three times, and then incubated in their own original plasma lose 95% of their passively acquired A or B antigen within 18 hours (Fig 3).

Group A₁ or B platelets maintained in A or B plasma, respectively, show no change in A or B antigen strength over 24 hours, but A₁ or B platelets incubated in buffer or absorbed O plasma demonstrate a decrease in reactivity during the same period. Most of this elution occurs in the first five hours of incubation, and by 24 hours, group A or B platelets still retain significant reactivity as compared with the essentially complete loss of antigens passively acquired on genetic group O platelets (Fig 4).

On two occasions we have extended this incubation of group A₁ platelets in O plasma to four days with daily changes of plasma. A maximum loss of 45% to 50% of A antigen was reached in approximately two days in group O plasma (Fig 4). In the same experiments there was no significant change in the level of type 2H antigen present on the group A₁ platelets (dotted line, Fig 4), as measured by a one-stage radioimmunoassay (RIA).

To determine the effect of plasma donor secretor status on the absorption of A and B antigens on to O platelets, the platelet suspensions were incubated for 24 hours in A and B plasma from known Lewis-type donors (Fig 5). The difference among the groups is significant when analyzed by Kruskall-Wallis test, although the difference between the Le(a+b-) and Le(a-b-) groups for A absorption is not significant when analyzed by a Wilcoxon two-sample test.
Le(a−b−) plasma donors were not tested for secretor status. However, the data suggest a pattern of antigen absorption for Le(a−b−)Se > Le(a−b+)Se > Le(a+b−)Se > Le(a+b−)Se as found previously by Kelton et al.2

DISCUSSION

Human platelets possess A and B antigens corresponding to the ABO blood group of the individual’s erythrocytes.1 4 These major blood group antigens can adversely affect the recovery and survival of transfused platelets if group O patients receive platelet concentrates from group A or B donors.2 4 Variability of response to ABO incompatible platelets may be due partly to differences in titer of immune anti-A and anti-B in the recipients and partly to differences in the amount of A or B antigen on donor platelets. The strength of A or B reactivity on platelets is affected by the Lewis and secretor type of the donor, and the amount of blood group A substance on the platelet is proportional to the amount of soluble type A substance in the plasma.2 Although earlier studies have evaluated the passive uptake of A and B substances from plasma to platelets, the contributions of intrinsic A and B antigen on platelets has not been previously assessed.

The major blood group ABH antigens are transferase-specified oligosaccharide determinants on glycoproteins or glycolipids.3 5 7 The terminal immunodeterminant sugars N-acetyl-galactosamine (A antigen) and D-galactose (B antigen) are attached in a β(1−3) linkage to the H determinant, which consists of β-D-galactose with an α-fucose at its two-carbon position. The β-D-galactose is attached to the oligosaccharide backbone by N-acetylgalcosamine. This linkage between β-D-galactose and N-acetylgalcosamine occurs in two forms, a β(1−3) linkage designated type 1 chain and a β(1−4) linkage termed type 2 chain. Thus the precursor H antigen and the derived A and B antigens exist either as type 1 or type 2 chains.5 7 In group A or B individuals, most of the A or B antigen on erythrocytes is synthesized by red cell precursors as type 2 glycolipids, whereas some and perhaps all ABH antigen passively acquired from the plasma consists of type 1 glycolipid.5 7 Because the H antigen serves as precursor for A and B antigens, the strength of H reactivity varies inversely with the amount of A or B antigen present on the cell, with intrinsic ABH structures composed of the β(1−4) type 2 chain.3 5 7

Our present study demonstrates that approximately 50% to 55% of ABH antigens on platelets occur as type 2H chains, which are presumably intrinsic, as when found on red cells, and the remainder occur as passively adsorbed structures, which are presumably type 1H chains of soluble glycolipid. The mouse monoclonal IgM anti-type 2H antibody has been characterized previously as specific for the type 2 configuration of intrinsic red cell H antigen.8 Direct assays using this anti–type 2H show dose-response saturation curves and the predicted variability in strength as an inverse function of substrate conversion, with H-negative (Oh) platelets demonstrating essentially no reactivity above indirect assay background values. If the platelet and red cell are assumed to be analogous with respect to derivation of type 2H structures, then our findings demonstrate that the human platelet possesses intrinsic antigens of the ABH system.

Our studies agree with those of previous workers on the passive uptake of soluble A antigen, which is probably a type 1 structure.2 4 Using the sensitive RIA,14 we have extended these observations to include the adsorption and elution of both A and B antigens on group O platelets, which is affected quantitatively by the Lewis and secretor type of the donor plasma. Furthermore, this rapid passive uptake appears completely reversible by returning the platelets to their original O plasma, indicating the gradual loss of the acquired A substance, presumably owing to elution from the platelet into the suspending plasma. By contrast, platelets from genetic A or B donors lost slightly less than half their reactivity when subjected to similar incubations, suggesting that a significant portion of their A or B antigens is intrinsic to the cell.

These data suggest that the ABH antigens on human platelets are a mixture of both intrinsic (type 2H) and extrinsic (type 1H) substances. Variations in the quantity of both adsorbed and intrinsic antigen may affect the outcome of ABO incompatible platelet transfusions, accounting for the irregular initial recoveries and distinct biphasic survival curves seen in some patients.2 4 Further work is needed for precise biochemical characterization of platelet ABH antigens and for assessment of the significance of intrinsic ν adsorbed antigens in platelet transfusion therapy.

REFERENCES


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