Expression of ABH and X (Le\(^\text{a}\)) Antigens on Platelets and Lymphocytes

By Rosella Mollicone, Thierry Caillard, Jacques Le Pendu, Anne François, Nicole Sansonetti, Henri Villarroya, and Rafael Oriol

We used a panel of reagents, polyclonal and monoclonal antibodies, and lectins to define the expression of the ABH-and Lewis-related specificities on platelets and lymphocytes. We also determined the expression of the \(\alpha_2\)- and \(\alpha_3\)-1-fucosyltransferases necessary for their biosynthesis. The antigens that could be detected by immunofluorescence and Western blot analysis were based on type 2 monofucosylated structures. Antibodies directed toward types 1, 3, and 4 ABH-, X- and Lewis-related antigenic determinants were always negative because the small amounts of ABH and Lewis antigens adsorbed from the serum could not be detected by these techniques. The presence of the type 2 ABH antigens on intrinsic glycoproteins was controlled by the \(\alpha_2\) gene. This correlates with the presence of \(\alpha_2\)-L-fucosyltransferase and the absence of \(\alpha_3\)-1-fucosyltransferase on platelets. In contrast, ABH antigens were not detected by immunofluorescence on normal peripheral lymphocytes. These cells thus have only the small amounts of antigens adsorbed from the serum, these being under control of the secretor and Lewis genes. This correlates with the absence of \(\alpha_3\)-L-fucosyltransferase on lymphocytes. When lymphocytes were transformed in vitro by the Epstein-Barr virus (EBV), however, they strongly expressed the X and sialylated X antigens, which are specific markers of normal granulocytes and monocytes, respectively. Treatment of EBV-transformed lymphoblastoid cell lines with 12-O-tetradecanoylphorbol-13-acetate significantly decreased the expression of X and sialylated X antigens along with that of surface immunoglobulins, whereas it induced a significant expression of the H antigen under control of the H gene.

The biosynthesis of the antigens based on the type 2 precursor is depicted in Fig 1. It involves two different fucosyltransferases, the \(\alpha_2\)-fucosyltransferase encoded by the H gene and the \(\alpha_3\)-fucosyltransferase encoded by the X gene. The action of the \(\alpha_2\)-fucosyltransferase is an absolute requirement for the biosynthesis of the A and B blood group antigens by the A and B glycosyltransferases. A similar biosynthetic pathway exists on the type 1 precursor that involves another \(\alpha_2\)-fucosyltransferase whose expression is controlled by the secretor gene not by the \(\alpha_2\) gene. This transferase competes for the same precursor substrate with the \(\alpha_4\)-fucosyltransferase encoded by the Lewis gene. The resultant products are the \(\text{Le}^\text{a}\) and \(\text{Le}^\text{b}\) antigens rather than the X and Y, their position isomers. On erythrocytes, the type 2 structures are borne by intrinsic components of the membrane, either glycoproteins or glycolipids, whereas the type 1 structures are only borne by glycolipids adsorbed from the plasma, and these come from tissues of endodermal origin.

It has been shown that human platelets and lymphocytes carry ABH and Lewis antigens as glycoconjugates adsorbed from the plasma. The expression of these adsorbed antigens is under the control of the Se and Le\(^\text{a}\) genes; however, it is not clear whether these cells also carry intrinsic ABH determinants as do erythrocytes. Using platelet-erythrocyte mixed agglutination, Kelton et al\(^\text{18}\) concluded that extrinsically adsorbed antigens were primarily responsible for the platelet ABH antigens; however, Dunstan et al\(^\text{19}\) using a radioimmunoassay concluded that adsorbed ABH antigens coexist with intrinsic determinants of platelets. It has also been reported that these intrinsic determinants should be borne by glycolipids rather than by glycoproteins. ABH determinants have never been shown to be present on intrinsic components of peripheral lymphocyte membranes; however, the glycosyltransferases responsible for the synthesis of ABH antigens have been detected in lymphocyte preparations.

The X antigen, also termed \(\text{Le}^\text{a}\) or SSEA1, corresponds to the trisaccharide \(\beta\text{Gal(1→4)[aFuc(1→3)]bGlcNAc}\). This structure has also a wide tissue distribution, but among blood cell types, only the granulocytes express it as revealed by monoclonal antibodies. The monococytes show a sialylated form of this antigen.

We studied the expression of these antigens on platelets, lymphocytes, and lymphoblastoid cell lines by using a panel of monoclonal and refined polyclonal antibodies and lectins of well defined specificities toward the various subtypes of ABH and X determinants. We also tried to correlate the antigenic expression to that of the \(\alpha_2\)- and \(\alpha_3\)-1-fucosyltransferases required for the biosynthesis of these antigens.

MATERIALS AND METHODS

Platelets

Ten to 20 mL of blood from healthy volunteers or donors of known ABO, Lewis, and secretor phenotypes was drawn into sodium citrate. Samples from blood group H-deficient individuals were obtained from Reunion Island. Both the “Reunion” phenotype, with a weak expression of H antigen, and the “Bombay” phenotype, with a complete lack of H antigen, were tested. Platelet-rich
plasma was prepared by centrifugation at 350 g for 15 minutes at room temperature and washed twice with 10 mmol/L Tris/HCl, pH 7.4, 154 mmol/L NaCl, and 1 mmol/L EDTA. The platelets were counted by using a Baker 810 platelet analyzer (Baker instrument Co, Allentown, PA).

**Mononuclear Cells**

Two different methods were used to prepare mononuclear cells.

**Method 1.** Twenty milliliters of blood was collected in heparin, and mononuclear cells were separated on Percoll. Briefly, 5 mL of 55% isotonic Percoll was layered over 5 mL of 74% isotonic Percoll and 5 mL of whole blood over this in a conical polystyrene tube. After centrifugation for 20 minutes at 350 g at room temperature, the upper ring containing the mononuclear cells was collected. The cells were then washed twice with phosphate-buffered saline (PBS; 10 mmol/L sodium phosphate, 0.14 mol/L sodium chloride, pH 7.2) by centrifugation at 700 g for 30 seconds.

**Method 2.** Forty milliliters of blood was collected on glass beads with constant agitation. The mononuclear cells were collected on Percoll as in method 1; however, this cell layer was contaminated with erythrocytes. To remove these cells, they were agglutinated by purified lectin from Galactia tenuiflora. This lectin binds to red cells and platelets but not to lymphocytes. After decantation of the agglutinates, the mononuclear cells were collected in the supernatant. The few remaining erythrocytes were lysed by the addition of 0.17 mol/L Tris-HCl, pH 7.2, and 0.16 mol/L NaCl.

**Anti–Blood Group A Reagents**

Monoclonal antibody 96A2 was obtained from Dr Rouger (Centre National de Transfusion Sanguine Institute, Paris). This antibody is known to react with monofucosylated type 1 and type 2 structures. Antibody 3-3A was obtained from Dr Bara (Villejuif, France). This antibody recognizes all types of A determinants: types 1, 2, 3, and 4 and difucosylated types 1 and 2. Antibody N16 was obtained from Dr Edelman (Pasteur Institute, Paris). It reacts primarily with A determinants, types 3 and 4. Antibody KB-26.5 was obtained from Dr Vinas (Knickerbocker, Barcelona, Spain). This antibody is specific for A determinants, types 3 and 4. Antibody TS-1, which has the same specificity as KB-26.5, was obtained from Dr Seitz (Hamburg, FRG). Antibody TT47A12 was obtained from Dr Terasaki (Los Angeles). It reacts specifically with difucosylated types 1 and 2 pentasaccharides (ALe– and AY).

**Anti–Blood Group B Reagents**

Antibody AAZ-137 is a purified polyclonal antibody preparation from goat antiserum. The antibodies were purified by affinity chromatography on synthetic B immunoadsorbent (B-Synorb, Chembiomed, Ltd, University of Alberta, Edmonton, Canada). Antibody b-183 was obtained from Dr Edelman (Paris); it behaves as an unrestricted anti-B reagent. Antibodies LA-4, KH-10, and LD-2 were obtained from Dr Jessell (Columbia University, New York). They react with nonfucosylated linear B antigen as well as with monofucosylated B epitopes. The polyclonal antibody preparation 143,62 was obtained by affinity chromatography of normal human serum on aGal(1→3)βGal-R immunoadsorbent. These purified antibodies do not react with the fucosylated blood group B antigens, but they react with the nonfucosylated pseudo-B antigen present on rabbit erythrocytes (Le Penu et al, unpublished).

**Anti–Blood Group H Reagents**

Antibody IIIT4 was obtained from Dr Seitz (Hamburg, FRG). This monoclonal antibody reacts specifically with the H type 2 and Y antigens. It does not react at all with type 1, 3, or 4 structures (Le Penu et al, unpublished). The polyclonal antibody 115,1 was obtained by affinity chromatography of goat antiserum. This antibody reacts strongly with H type 2, H type 1, and Y determinants. Ulex europaeus lectin 1 (UEA 1), affinity purified and labeled with fluorescein isothiocyanate (FITC) was obtained from Vector Laboratories, Inc, (Burlingame, CA). Galactia tenuiflora lectin (GTA), affinity purified and FITC labeled, was prepared as previously described. This lectin is specific for H type 2 determinants.

**Anti–Lewis–Related Reagents**

Antibody CA-50 was obtained from Dr Svennerholm (Göteborg, Sweden). This antibody binds to the sialylated type 1 precursor and Lea antigens. Antibody 2-25LE was obtained from Dr Bara (Villejuif, France). This monoclonal antibody reacts with both Lea and Leb antigens. The polyclonal, affinity-purified antibody RO-23 reacts specifically with the Leb antigen. Monoclonal antibody AH7-105 is an anti-Leb-specific antibody obtained from Chembiomed. Antibody 80H5 was obtained from Dr Mannoni (Marseille, France) it reacts specifically with the X or Lea trisaccharide. Antibody 101 was obtained from Dr Richter (Bethesda, MD); it is specific for the Y or Leb tetrasaccharide. Antibody CSLEX 1 was obtained from Dr Terasaki (Los Angeles) and is specific for sialylated X or Leb determinants.

**Antiplatelets**

An antiplatelet glycoprotein (Gp) IIb-IIIa monoclonal antibody was obtained from Immunotech (Marseille, France).
**Immunofluorescence**

For each assay, about 3 x 10^7 platelets/50 μL or 10^6 mononuclear cells/50 μL was used. To these cells was added 50 μL of the antibodies or lectins at the appropriate dilutions. These mixtures were incubated for 30 minutes at room temperature. Optimal dilutions of all reagents were determined by chessboard titrations. Affinity-purified antibodies were used at concentrations ranging from 5 to 20 μg/mL, whole ascitic fluids at dilutions from 1/100 to 1/1,000, and hybridoma supernatants diluted 1 in 2. All dilutions were made in 1% bovine serum albumin (BSA) in PBS. The cells were then washed three times with PBS. When FITC-labeled lectins were used, the fluorescence was examined directly. When antibodies were used, a second incubation step was performed by using FITC-labeled antimouse, antihuman, or antigoat immunoglobulins (Pasteur Institute). After three more washings, fluorescence was observed under a Leitz SM-LUX microscope (Leitz, FRG) equipped with a Plomopak 2.3 and a lamp source of 200 W mercury vapor lamp.

On lymphoblastoid cell lines, the expression of surface immunoglobulins was studied by using purified antihuman FITC-labeled immunoglobulins (Pasteur Institute).

**Purification of Monoclonal Antibodies**

Antibodies 3-3A and IIIT4, which were used in the immunoblot experiments, were purified by affinity chromatography on synthetic oligosaccharides coupled to an insoluble matrix. These Synsors were obtained from Chembromed. Five hundred microliters of crude ascitic fluids was loaded into columns containing 1.5 g of immunoadsorbents. These were A synsors for 3-3A and H type 2 synsor for IIIT4. After extensive washing of the columns, the adsorbed antibodies were eluted with 2% NH₄OH and 0.15 mol/L NaCl and immediately neutralized with a solution of saturated KH₂PO₄. After dialysis against PBS, the purity of the antibodies was checked by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE).

**Immunoblot of Platelet Glycoproteins**

Whole platelets were solubilized in 30 mmol/L Tris-HCl, pH 7.2, 3 mmol/L EDTA, and 6% SDS. After reduction in 5% β-mercaptoethanol, the solubilized platelets were subjected to electrophoresis in 10% polyacrylamide gels according to the method of Laemmli. The separated proteins and glycoproteins were electrophoretically transferred to nitrocellulose filters in 25 mmol/L Tris and 0.192 mol/L glycine, pH 8.3, at 70 V for two hours. The nitrocellulose filters were then incubated for one hour at 37°C in 5% BSA in PBS. The purified anti-A (3.3A) and anti-H (IIIT4) antibodies were then incubated overnight at room temperature. The antibodies were diluted in 3% BSA in PBS at 0.1 and 2 μg/mL, respectively. After three washes in PBS for ten minutes each, the filters were incubated with peroxidase-labeled antimouse immunoglobulins (Pasteur Institute) for three hours.

After three more washings, the bound peroxidase was revealed with diaminobenzidine (DAB) enhanced with 1.25 mmol/L CoCl₂ (6 mg CoCl₂ plus 10 mg DAB in 20 mL PBS with 0.1 mL H₂O₂, 110 vol).

**Lymphoblastoid Cell Lines**

Epstein-Barr virus (EBV) infection of peripheral lymphocytes from healthy individuals of known H and secretor genotypes was carried as previously described. Briefly, 100 μL previously frozen whole blood was mixed with 1 mL virus-containing supernatant; 2.5 mL RPMI containing 20% FCS was added to a 25 cm² culture flask.

The cells were incubated at 37°C in a 5% CO₂ atmosphere, and each week 2 mL of medium was added to the cultures. The cell lines thus obtained were then cultured in RPMI 1640, 10% FCS with or without 12-0-tetradecanoylphorbol-13-0-acetate (TPA) at 2 μg/mL. After two days in culture, immunofluorescence assays were performed. More than 90% of the cells were viable.

**Fucosyltransferase Assays**

The reaction mixture of the H α-2-L-fucosyltransferase contained, in a total volume of 130 μL, 1% Triton X-100 cell lysates (5 x 10⁶ cells); Tris-HCl, pH 7.2, 1.0 μmol; MgCl₂, 1.0 μmol; adenosine triphosphate (ATP) 0.25 μmol; guanosine diphosphate (GDP) [¹⁴C] fucose (210 μCi/μmol, NEN Chemicals, Frankfurt, FRG), 620 pmol; and phenyl-β-D-galactoside, 0.16 μmol (Koch-Light Laboratories, Colnbrook Berks, UK). After incubation for 64 hours at 37°C, the reaction products were separated by paper chromatography in ethyl acetate/pyridine/water, 10:4:3. The [¹⁴C] labeled products were localized on a radiochromatogram scanner (Packard 7201, Downers Grove, IL) and quantified by liquid scintillation counting. The product was characterized by its chromatographic mobility with fucose as a reference (Rfuc, 1.5).

The reaction mixtures for the α-3-L-fucosyltransferase were similar except that MgCl₂ was replaced by MnCl₂, 1.0 mmol, and phenyl-β-D-galactoside by N-acetyllactosamine, 0.23 μmol (Sockerbolaget, Arlöv, Sweden). The products were separated in the same solvent. The specific product was characterized by its mobility with lactose as a reference (Rlac, 0.75).

**RESULTS**

**Immunofluorescence**

**Platelets.** As shown in Table 1, all platelets were stained by some anti-ABH reagents according to the donor's ABO phenotype. Staining was not influenced by the secretor and Lewis phenotypes of the individual. Staining of platelets from H-deficient individuals was never observed. Only those reagents recognizing type 2 monofucosylated structures were positive. The reagents specific for difucosylated type 1 or type 2 structures were always negative (TT47A12, 101, RO-23). Anti-A, types 3 or 4–specific antibodies were also negative (N16, KB-26.5, TS-1). Anti-Lewis antibodies were negative including the anti-X or Le₃ and sia1ylated X reagents (80H5 and CSLEX1). Only antibody CA-50 strongly stained a small population of platelets of all the individuals tested.

**Mononuclear cells.** The anti-A, -B, or -H antibodies never stained mononuclear cells. Anti-Lewis–related antibodies were also negative on these cells except for antibody CSLEX1, which binds to monocytes; however, when the mononuclear cells were prepared by method 1 as described in Materials and Methods, many contaminating platelets could be identified with the anti-monofucosylated type 2 anti-A, -B, or -H reagents and by anti-GpIb-IIIa platelet glycoprotein. Some of these contaminating platelets were bound to the mononuclear cell membranes. When the mononuclear cells had been prepared by method 2, no fluorescence with anti-ABH reagents could be revealed in the preparation. Anti-GpIb-IIIa antibody was also negative, thus confirming that these mononuclear cell preparations were completely free of platelets.
Table 1. Immunofluorescence on Normal Adult Platelets With Antibodies or Lectins

<table>
<thead>
<tr>
<th>Anti-A</th>
<th>Anti-B</th>
<th>Anti-H</th>
<th>Anti-Lewis</th>
</tr>
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<tbody>
<tr>
<td>96A2</td>
<td>++ +</td>
<td>AAZ-137</td>
<td>++ +</td>
</tr>
<tr>
<td>3-3A</td>
<td>++ +</td>
<td>b-183</td>
<td>++ +</td>
</tr>
<tr>
<td>N16</td>
<td>-</td>
<td>LA-4</td>
<td>++ +</td>
</tr>
<tr>
<td>TT47A12</td>
<td>-</td>
<td>KH-10</td>
<td>-</td>
</tr>
<tr>
<td>KB-26.5</td>
<td>-</td>
<td>LD-2</td>
<td>-</td>
</tr>
<tr>
<td>TS-1</td>
<td>-</td>
<td>143.62</td>
<td>-</td>
</tr>
</tbody>
</table>

In the positive reactions, all or almost all platelets were strongly fluorescent. Anti-A antibodies were positive on blood group A and AB platelets, anti-B antibodies on blood group B and AB platelets, and anti-H reagents on A, B, AB, or O platelets except O, Bombay or Reunion phenotypes. The polyclonal antibody 115,1 strongly stained a major band around 140 kd. Another band around 130 kd was also strongly detected by anti-A antibody. Weaker bands ranging from 160 to 60 kd were also labeled.

Lymphoblastoid cell lines. The five lymphoblastoid cell lines tested expressed the X and sialylated X antigens, although the percentage of positive cells varied from one cell line to another and from one experiment to another. These variations should be due to the dynamics of antigen expression on cells as a function of time because the technical variation in the assay itself is ±10% (Table 2).

The number of positive cells always decreased after treatment with TPA. The number of surface immunoglobulins also decreased after treatment with TPA.

Table 2. Immunofluorescence on EBV Lymphoblastoid Cell Lines

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Genotype</th>
<th>Without TPA</th>
<th>With TPA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>X</td>
<td>Si-X</td>
</tr>
<tr>
<td>29930</td>
<td>H/H, Se/se</td>
<td>39</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>33</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>20</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>43</td>
<td>16</td>
<td>-</td>
</tr>
<tr>
<td>107977</td>
<td>H/h, se/se</td>
<td>12</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>76</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>107965</td>
<td>H/h, se/se</td>
<td>60</td>
<td>18</td>
</tr>
<tr>
<td>29932</td>
<td>h/h, se/se</td>
<td>68</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>57</td>
<td>23</td>
<td>-</td>
</tr>
<tr>
<td>107932</td>
<td>h/h, se/se</td>
<td>39</td>
<td>19</td>
</tr>
</tbody>
</table>

Cells were cultured in RPMI 1640 with 10% FCS; TPA was added (2 μg/mL) in some cultures, and the immunofluorescence assays were performed after two days of culture. The internal variation of the test measured on nine replicates was ±10%. The X antigen was detected with antibody 80H5, its sialylated form (Si-X) with antibody CSLEX1, the H antigen with the affinity-purified polyclonal antibody 115,1, and the surface immunoglobulins (Ig) with polyclonal anti-human immunoglobulins. A comparison of the results for each experiment, with and without TPA, was performed by the paired t test: a decrease in the antigen X content, P < .001 (n = 10); a decrease in the sialylated-X content, P < .02 (n = 9); an increase in the antigen H content in H/H individuals, P < .0001 (n = 5); and a decrease in the number of surface immunoglobulins, P < .003 (n = 10).

Normally, the H antigen is not or is very weakly expressed on lymphoblastoid cell lines; however, after treatment with TPA a significant proportion of cells expressed this antigen very strongly as revealed by using the refined polyclonal antibody 115,1. This antigen could not be expressed in the cell lines originating from two H-deficient individuals, whereas it was seen in the cell line from a nonsecretor individual, which indicates that it is the H gene product that can be expressed in the lymphoblastoid cell lines.

Immunoblot of Glycoproteins from Platelets

After transfer to nitrocellulose filters, some platelet glycoproteins could be specifically stained with affinity-purified anti-A and anti-H monoclonal antibodies (Fig 2). The anti-A antibody strongly stained a major band around 140 kd. Another band around 130 kd was also strongly detected by this antibody on platelets of blood group A individuals. Weaker bands ranging from 160 to 60 kd were also labeled.

The anti-H antibody was weaker, but the staining pattern was essentially the same. The major band was around 140 kd. Another band at 160 kd was also visible; however, nothing appeared at 130 kd as with the anti-A antibody. Very faint bands were also visible as low as 60 kd. The staining produced by this antibody was stronger on O platelets than on A platelets, and no staining at all was visible on O platelets, thus confirming the blood group H specificity of this labeling. It thus appears that the major band stained with the anti-A and anti-H antibodies could be Gplbo.

To rule out the possibility that the various glycoproteins stained with the antibodies could have been adsorbed from the serum, we incubated platelets from a blood group O person in the serum of a blood group A individual for one hour at 37°C in the presence of protease inhibitors. After SDS-PAGE and transfer to the nitrocellulose filter of the separated glycoproteins, no staining was detectable with the anti-A antibody, whereas normal staining was retained with anti-H antibody (data not shown).
ABH AND X ON PLATELETS AND LYMPHOCYTES

Fig 2. Biochemical characterization of the platelet glycoproteins carrying ABH determinants. Solubilized platelets were reduced in 5% β-mercaptoethanol and submitted to SDS-PAGE and Western blot. Lanes 1 and 2, Chinese ink staining of total proteins transferred to the nitrocellulose filter from blood group O and A platelets, respectively; lanes 3 and 4, immunostaining with anti-A (3-3A) on O and A platelets extracts; lanes 5, 6, and 7, immunostaining with anti-H (III T4) on O, A, and O, (Bombay) platelets. Molecular weight markers are indicated in kilodaltons.

Fucosyltransferase activities

Two different methods of preparation of mononuclear cells were used. When blood was collected in heparin as in method 1, the mononuclear cells were always strongly contaminated by platelets, as observed by immunofluorescence using the anti–GpIIb-IIIa antibody, which specifically stains the platelets. In this case as shown in Table 3, a strong α-2-L-fucosyltransferase was found along with a strong α-3-L-fucosyltransferase. When blood was collected on glass beads and the few remaining platelets and erythrocytes were removed by agglutination with an anti-H lectin as in method 2, no α-2-L-fucosyltransferase activity could be detected, whereas a strong α-3-L-fucosyltransferase activity remained, although it varied between the three individuals tested. This clearly shows that the α-2-L-fucosyltransferase activity detected comes from the platelets and not from the mononuclear cells, which have the α-3-L-fucosyltransferase activity. When using the trisaccharide αL-Fuc(1→ 2) βGal(1→ 4)βGlcNAc-O-(CH2)3-CO-CH3 as an acceptor, the platelets did not have this α-3-L-fucosyltransferase activity at all (data not shown).

DISCUSSION

We have shown by immunofluorescence that platelets carry ABH antigenic determinants under control of the H gene and independently of the secretor and Lewis characters. These determinants were only revealed by the antibodies that could recognize type 2 monofucosylated structures. All the reagents specific for type 1, types 3 or 4, and type 2 difucosylated structures did not stain platelets. The only exception concerned the antigen CA-50, a Lewis-related determinant that was strongly expressed on a small population of platelets from all the individuals tested. It is thus likely that the very small amounts of Lewis antigens present on platelets that are adsorbed from the plasma cannot be detected by our immunofluorescence method.

The immunoblot experiments confirmed that the detected ABH antigens were not adsorbed from the plasma and showed that a variety of platelet glycoproteins do carry ABH determinants. The major glycoprotein thus revealed comigrated with GpIIb. Because the fragment of this glycoprotein, called glycocalcin, is known to be highly O-glycosylated, it is likely that it is this glycoprotein that contributes to the major part of the glycoprotein-bearing ABH determinants of platelets. It has also been suggested, however, that glycolipids of intrinsic origin do carry ABH determinants.

Therefore, platelets, like erythrocytes, carry two families of ABH determinants: (a) those adsorbed from the plasma that appear to be mostly based on the type 1 precursor and are under the control of the Se and Le genes and (b) intrinsic determinants carried by both glycoproteins and glycolipids. This is in accordance with the fact that platelets, like erythrocytes, show an α-2-L-fucosyltransferase activity as well as the α-N-acetylgalactosaminyl and α-galactosyltransferases responsible for the synthesis of blood groups A and B, respectively.

Although anti-A and -B antibodies have been shown to be responsible for the refractoriness of platelet transfusions in patients with high antibody titers, usually ABO-incompatible platelet transfusions only result in a small reduction in platelet recovery. This important difference between erythrocytes and platelets could be due not only to the lower total amount of ABH antigens on platelets but also to the fact that

Table 3. α-2- and α-3-L-Fucosyltransferases in Mononuclear Cells

<table>
<thead>
<tr>
<th>Method</th>
<th>α-2-Fucosyltransferase</th>
<th>α-3-Fucosyltransferase</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14,348</td>
<td>84,504</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>122,760</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>19,851</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>33,326</td>
</tr>
</tbody>
</table>

The reaction mixtures for α-2-L-fucosyltransferase contained, in a total volume of 130 μl, 1% Triton X-100 cell lysates (5 × 10⁶ cells); Tris-HCl, pH 7.2, 1.0 μmol; MgCl₂, 1.0 μmol; ATP, 0.25 μmol; GDP[¹⁴C]fucose, 620 pmol; and phenyl-β-D-galactoside, 0.16 μmol. The reaction mixtures for α-3-L-fucosyltransferase were similar except that MgCl₂ was replaced by MnCl₂, 1 μmol, and phenyl-β-D-galactoside by N-acetylgalactosamine, 0.23 μmol. The mixtures were incubated for 64 hours at 37°C, and the products were separated by paper chromatography in ethyl acetate/pyridine/water, 10:4:3, and characterized by their chromatographic mobilities. Mononuclear cells prepared by method 1 (see Materials and Methods) were contaminated by platelets as revealed by immunofluorescence using anti-GpIIb-IIIa platelet glycoprotein. Mononuclear cells from three different individuals prepared by method 2 were free of platelets.
the highly immunogenic types 3 and 4A antigens, specific for blood group A1 erythrocytes, are absent from platelets. We have also shown that platelets do not have the α-3-L-fucosyltransferase responsible for the synthesis of the X or Leα determinant. This correlates with the absence of this antigen and its fucosyl (Y) and sialyl derivatives.

ABH and Lewis determinants, under the control of the Se and Le genes, have been detected on lymphocytes by using lymphocytotoxicity assays. These antigens are adsorbed from the plasma and appear to be primarily of type 1, although that could be due to the lack of cytotoxic antibodies. These antigens are adsorbed by lymphocytes, although they have been observed in small amounts by fluorescence flow cytometry. Our technique is probably not sensitive enough to detect these absorbed antigens. It was also possible to show that the previously reported expression of the α-2-L-fucosyltransferase on lymphocytes was in fact due to a contamination of these cells by platelets. Thus, the absence of intrinsic ABH determinants on lymphocytes correlates with the absence of the glycosyltransferase necessary for their synthesis; however, as previously reported, a strong α-3-L-fucosyltransferase activity was present in the mononuclear cell preparations. These preparations contained lymphocytes and monocytes. These last cells express the sialyl X antigen. Therefore, we cannot decide at present whether the α-3-L-fucosyltransferase that we detected originated from lymphocytes or from monocytes.

When peripheral lymphocytes were transformed with EBV, they did express the X and sialylated X antigens, which are normally considered to be markers of granulocytes and monocytes, respectively. These lymphoblastoid cell lines could also express the H antigen when cultured with TPA. Under the same conditions, X or Leα antigen expression always decreased in parallel with expression of the surface immunoglobulins. Absent expression of these antigens can thus be observed after viral transformation.

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28. Huang LC, Civin CI, Magnani JL, Shaper JH, Ginsburg V: My-1, the human myeloid-specific antigen detected by mouse monoclonal antibodies, is a sugar sequence found in lacto-N-fucopentaose III. Blood 61:1020, 1983


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