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Glycolipid- and Glycoprotein-based Blood Group A Antigen Expression in Human Thrombocytes. A_1/A_2 Difference

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Total non-acid glycolipid fractions and total sodium dodecylsulphate (SDS) solubilized protein fractions were isolated from human thrombocytes obtained from single human donors having different blood group A_1/A_2 phenotypes. The blood group A glycolipid antigens were characterized by immunostaining of thin layer plates with different monoclonal anti-A antibodies. The glycoproteins carrying blood group A epitopes were identified by SDS-PAGE and Western blot analysis using a monoclonal anti-A antibody. Blood group A glycolipid antigens were found in both A_1 and A_2 thrombocytes but the A_2 individuals expressed at least ten times less A glycolipids compared to the A_1 individuals. Expression of A type 3/4 chain and small amounts of A type 1 chain glycolipids were seen in thrombocytes of both A_1 and A_2 individuals, while the type 2 chain A glycolipids appeared to be missing from the A_2 thrombocytes. Blood group A reactive glycoproteins were only found in thrombocytes of A_1 individuals and could not be detected in A_2 individuals or a blood group O individual. The major blood group A glycoprotein were found as a double band migrating in the 130 kDa region.

Blood group ABH antigens in thrombocytes have been claimed to be both endogenously synthesized and adsorbed from plasma [1-3] and have been shown to exist both as glycosphingolipids [3] and glycoproteins [3]. It is known that blood group ABO compatibility

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Abbreviations: SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; HPTLC, high performance thin layer chromatography; CBB, Coomassie brilliant blue; GVH, graft versus host. In the short hand designation for glycolipids, the letter indicate blood group determinant, the first numeral, the number of sugar residues, and the second numeral, the type of carbohydrate chain. Thus, A-6-1 means a hexaglycosylceramide with a blood group A determinant based on the type 1 carbohydrate chain.

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can influence the survival of transfused thrombocytes and that patients with high anti-ABO antibody titers may experience poor increments of thrombocyte counts upon blood group ABO incompatible thrombocyte transfusions [4]. The IgG antibodies of patients having high anti-A titers have by immunofluorescence been shown to react with blood group A_1 thrombocytes but not with blood group A_2 thrombocytes [5]. The latter also poorly absorbed a mouse monoclonal anti-A antibody [5].

In this paper, we compare the expression of glycolipid- and glycoprotein-bound blood group A antigens in blood group A_1 and A_2 thrombocytes. The findings are discussed in relation to the significance of ABO compatibility in thrombocyte transfusions.

Experimental Procedures

Preparation of Cells

Thrombocytes were isolated according to standard blood banking procedures either by thrombocytapheresis or from ordinary blood units. The donors were blood grouped on their erythrocytes and in some cases the secretor status was established by saliva analysis. The contamination of leucocytes in the thrombocyte preparations did not exceed 0.05%.

Glycolipid Preparation

Total non-acid glycolipid fractions were isolated from thrombocytes obtained by thrombocytapheresis of a blood group A_1Le(a-b+)Secretor (about 300 x 10^9 cells) and A_2Le(a-b+) Secretor (about 200 x 10^9 cells) individual mainly as described [6]. The preparation procedure was identical for both fractions.

Analytical Thin Layer Chromatography

Thin layer chromatography was performed on HPTLC plates (Merck, Darmstadt, FRG) and the solvent used was chloroform/methanol/water, 60/35/8 by vol. Detection was accomplished by a chemical reagent, anisaldehyde [7] and by immunostaining of the plates by monoclonal antibodies followed by autoradiography [8, 9]. Mouse monoclonal anti-A antibodies reacting with all structurally different A antigens were obtained from Dakopatts (Glostrup, Denmark; code A581) [10] and Biotest diagnostics (Frankfurt am Main, FRG; Seraclone anti-A). The mouse monoclonal KB-26.5 antibody reacting with blood group A antigens based on type 3 [GalNAcα1-3(Fucα1-2)Galβ1-3GalNAcα-] and type 4 [GalNAcα1-3(Fucα1-2)Galβ1-3GalNAcβ-] chains was obtained from Knickerbocker (Barcelona, Spain) [11]. The A type 1 [GalNAcα1-3(Fucα1-2)Galβ1-3GlcNAcβ-] [12] and A type 2 [GalNAcα1-3(Fucα1-2)Galβ1-4GlcNAcβ-] [13] chain specific antibodies (AH21 and HH4, respectively) were kindly provided by Drs. H. Clausen and S. Hakomori. Pure reference blood group A glycolipids based on type 1 (A-6-1) [14], type 2 (A-6-2) [15], and type 4 (A-7-4) [16] chains were isolated from various tissues as described.

Sodium Dodecylsulphate-Polyacrylamide Gel Electrophoresis and Western Blotting

SDS-PAGE was performed on thrombocytes heated to 100°C for 2 min in 6% SDS. SDS-PAGE was performed with a 3% stacking gel and a 7% acrylamide resolving gel using a vertical electrophoresis unit (LKB 2001, Pharmacia-LKB, Sweden) essentially as described [17]. Protein staining was accomplished by Coomassie Brilliant Blue (CBB). Separated proteins were electrophoretically blotted onto the nitrocellulose membrane using a semi-dry electroblotting apparatus (LKB 2117-005 Multiphor II Novablot, Pharmacia-LKB, Sweden) [18]. Proteins bearing the blood group A determinant were detected by monoclonal anti-A antibodies from Biotest (see above) followed by a secondary radioactively labeled antibody essentially as described [19]. The sample protein concentration was determined by the BCA protein assay reagent according to the instructions from the manufacturer (Pierce Chemical Company, Rockford, IL, USA).

Results

Glycolipid Analysis

The non-acid glycolipids from thrombocytes were dominated by mono- to tetraglycosylceramides, with only quantitative differences between the A_1 and A_2 individuals (Fig. 1A, lanes 3, 4, 5, and 6). The A_2 individual seemed to express less tri- and tetraglycosylceramides than the A_1 individual (compare lanes 3 and 5). Compared to the non-acid glycolipids from the erythrocytes (lane 1) and plasma (lane 2), the thrombocyte-derived glycolipids seemed to contain less amounts of components in the blood group interval migrating below the four-sugar region on the thin layer plate. A very complex pattern of blood group A glycolipids was revealed by immunostaining the plates with various monoclonal anti-A antibodies (Fig. 1B-E). When antibodies specific for the A trisaccharide (B), the type 2 chain A (D), and the type 3 and 4 chain A antigens (E) were used, the glycolipid thin layer patterns of erythrocytes and plasma were clearly different from the thrombocyte glycolipid pattern. However, the anti-A type 1 chain specific antibody (C) gave weak staining in the six sugar region in the plasma fraction (lane 2) as well as in the thrombocyte fractions, although clearly seen only in those fractions where five times the amount was applied (lanes 4 and 6, respectively). The thrombocytes showed blood group A reactivity in the six, eight/nine, and ten/twelve sugar regions with the anti-A antibody recognizing the terminal A trisaccharide (B), and in the eight/nine and ten/twelve sugar regions with the anti-A type 3 and 4 chain antibody (E). The A_2 individuals expressed at least ten times less glycolipid-bound A antigens (compare lanes 3 and 6, plate B) and appeared to lack or to express only trace amounts of type 2 chain A glycolipid antigens (compare lanes 3 and 4 with lanes 5 and 6, plate D).

Glycoprotein Analysis

The total SDS-solubilized protein fractions from the thrombocytes were very similar irrespective of blood group phenotype upon SDS-PAGE analysis and CBB staining (Fig. 2, top). However, the monoclonal anti-A antibody (Seraclone) used in Western blot experiments revealed a clear difference between A_1 and A_2 individuals. A major double band around 130

Fig. 1

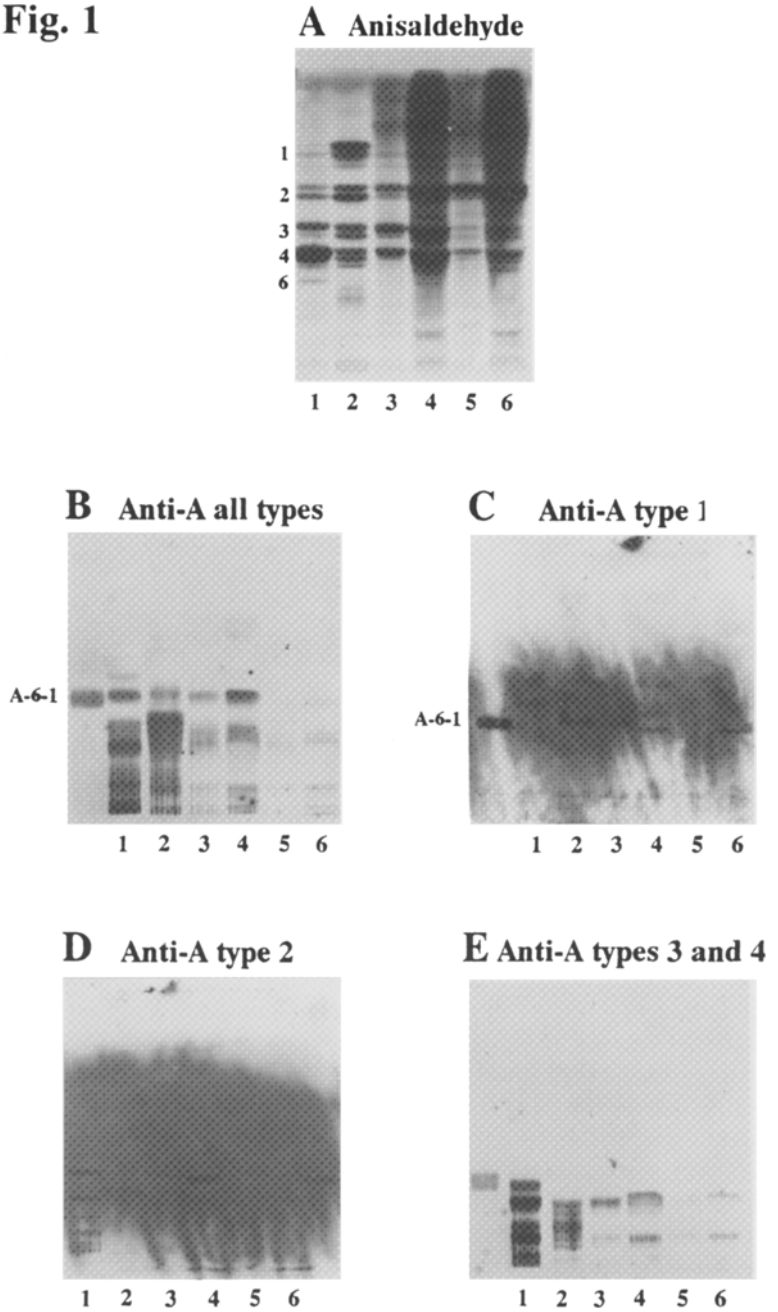

Figure 1. The thin layer chromatographic analysis of the total non-acid glycolipids isolated from thrombocytes of a blood group A_1Le(a-b+)Secretor (lanes 3 and 4) and an A_2Le(a-b+)Secretor (lanes 5 and 6) human individual, together with the non-acid glycolipids of erythrocytes (lane 1) and plasma (lane 2) of blood group A_1Le(a-b+) individuals. The amount of sample applied was 40 µg in lanes 1 and 2, 20 µg (1/115 of the fraction) in lane 3, 20 µg (1/100) in lane 5, and 100 µg in lanes 4 and 6, respectively. Detection was accomplished by anisaldehyde (chromatogram A) and by autoradiography after immunostaining the thin layer plates with mouse monoclonal antibodies specific for the terminal A trisaccharide (B), the type 1 chain monofucosyl A (C), the type 2 chain monofucosyl A (D), and the type 3/4 chain A antigen (E). Appropriate blood group A reference glycolipids (A-6-1, A-6-2, and A-7-4) was applied (0.2 mg) on each plate.

kDa, as estimated by linear extrapolation from the weight standard at 94 kDa, and several less stained bands were seen in the blood group A_1 individuals, while no staining was seen among blood group A_2 individuals or the blood group O individual used as a negative control (Fig. 2, bottom). Even at sample protein concentrations fifty times that applied for the A_1 individuals, no staining was seen among the blood group A_2 individuals (not shown). Separate thrombocyte protein preparations from additionally twenty A_2 individuals gave the same result (not shown). No correlation to the secretor phenotype was seen in the expression of protein-bound blood group A epitopes (Fig. 2, compare lanes 1 and 2 with lanes 3 and 4).

Discussion

There have been conflicting data in the literature concerning the origin of the ABH antigens in thrombocytes. Papers in favour of the ABH antigens being mainly extrinsically adsorbed [2] as well as both extrinsically adsorbed and endogenously biosynthesized [1, 3] have been presented. From our results it can be concluded that the thrombocyte-derived glycolipids can not exclusively originate from passively adsorbed plasma glycolipids, since the glycolipid-staining pattern was clearly different in these tissues (Fig. 1B and E, lanes 2 and 3). The most intensely stained component seen with the anti-A type 3/4 antibody in the ten/eleven sugar region in the plasma fraction was absent in the thrombocyte fraction (Fig. 1E, lanes 2 and 3). However, this finding does not exclude passively adsorbed blood group ABH glycolipids. The weak staining seen in the six sugar region using the monoclonal anti-A type 1 chain antibody, may be due to a blood group A monofucosyl type 1 chain hexaglycosylceramide structure (Fig. 1C, lanes 4 and 6). This structure is known to be present in plasma [20] and to be dependent of the *Se* gene [21]. The tentative presence of this structure in the thrombocytes, which are supposed to lack the *Se* gene dependent α-2-L-fucosyltransferase [3], may indicate a passive adsorption from plasma.

No type 1, 3 or 4 chain A structures were identified in a previous study by the use of immunofluorescence and Western blot techniques [3]. However, the type 3 and 4 chain A specific antibody clearly stained thrombocyte glycolipids in the eight/nine and ten/twelve sugar regions (Fig. 1E, lanes 3 to 6). A weak staining was also seen with the type 1 chain A specific antibody (see above).

The A_2 individual expressed at least ten times less lipid-bound blood group A antigens (Fig. 1B, lanes 3 and 6) and lacked or had only trace amounts of type 2 chain A glycolipid antigens (Fig. 1D, lanes 5 and 6). Furthermore, no protein-bound blood group A determinants were found in the A_2 thrombocytes, even at protein concentrations fifty times as high as those giving clear staining in the blood group A_1 thrombocytes (Fig. 2, bottom and experiments not shown). Several additional A_2 individuals were investigated without any staining being seen in the Western blot analysis of their thrombocyte proteins. However, all blood group A_1 individuals have shown reactivity in the 130 kDa region, whereas the most intensely stained component found by Oriol *et al.* migrated in the 145 kDa region [3]. Nothing is known about the core saccharide chain carrying the A determinant and whether the A determinant is located on N- or O-linked carbohydrate chains. However, if one assumes that the protein-bound blood group A determinant is located on a N-linked sugar chain, it is most likely carried by a lactosamine backbone (type 2 chain) [22, 23]. Oriol *et al.* were only able to

Fig. 2

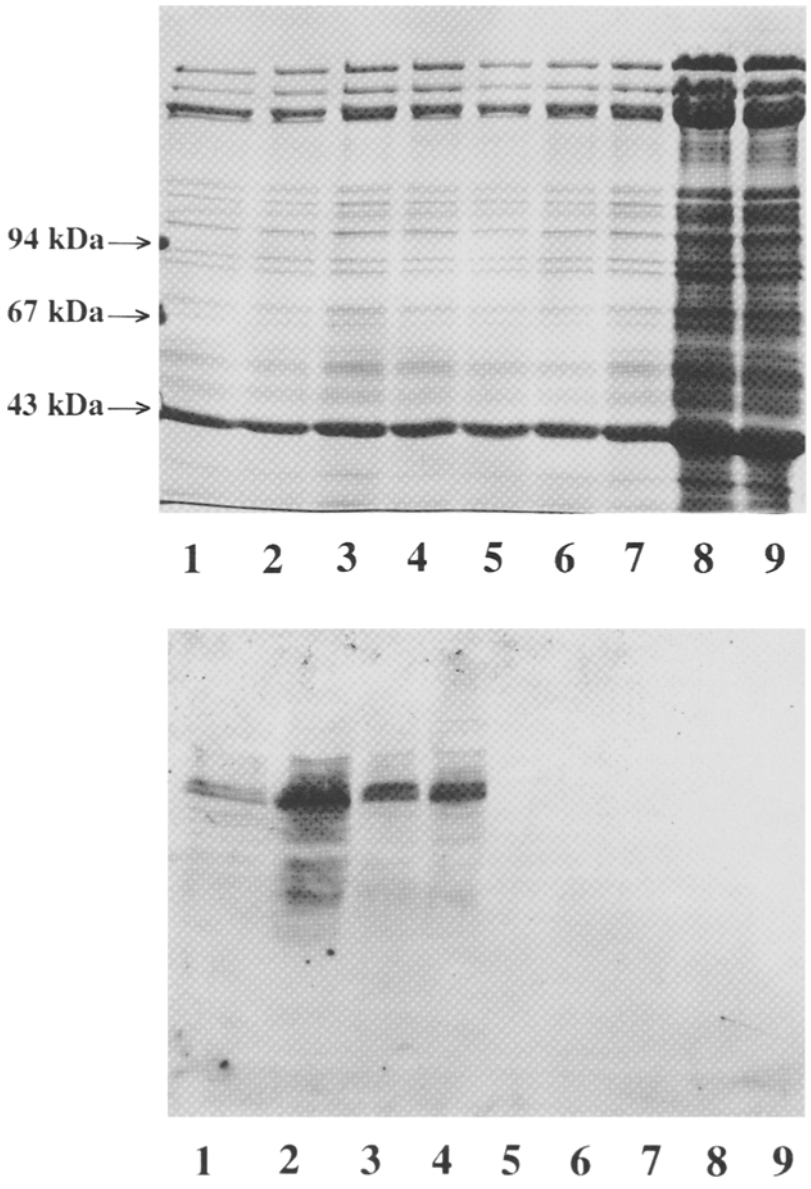

Figure 2. SDS-PAGE analysis of total SDS-solubilized proteins from thrombocytes obtained from blood group A_1Le(a+b-) (lanes 1 and 2), A_1Le(a-b+) (lanes 3 and 4), A_2Le(a-b+) (lane 5), A_2Le(a-b-) (lane 6) individuals. A blood group O individual (lane 7) was used as a negative control. About 75 µg protein was applied in each slot (lanes 1 to 7). The same samples as in lanes 5 and 6 were applied in lanes 8 and 9, respectively, but at a concentration ten times as high. Detection was accomplished by Coomassie Brilliant Blue (top) and autoradiography after Western blot with a monoclonal anti-A antibody reacting with the terminal A trisaccharide (Seraclone, Biotest, FRG) (bottom).

identify type 2 chain A structures on thrombocytes from blood group A donors [3], which might be explained by an inability, in certain cases, of the immunofluorescence method to detect epitopes close to the membrane bilayer (i.e. type 1, 3 and 4 chain A glycolipids). That is, the A_2 thrombocytes seem to lack or to have only trace amounts of type 2 chain A antigens (both glycolipid- and protein-bound). The type 2 chain antigens are known to be endogenously synthesized in human erythrocytes [21] and can be expected to be so also in thrombocytes according to their embryological origin [21]. It may though be, that the A_2 thrombocytes lack or have only minor amounts of endogenously synthesized A antigens, which accounts for the difference between the A_1 and A_2 thrombocytes.

The lack of protein-bound A determinants among A_2 thrombocytes together with the expression of less glycolipid-bound A antigens might be the molecular explanation for the successful transfusion of A_2 thrombocytes to patients having high anti-A titers and responding poorly to A_1 thrombocyte transfusions [5]. The present data might also be of clinical relevance in bone marrow transplantation situations when the recipients are given thrombocyte transfusions. Blood group A_2 thrombocytes would be the choice in situations where a blood group A recipient has received an O bone marrow graft. Transfused A_2 thrombocytes would not suffer from a severe GVH rejection and no passenger anti-A Ig would cause tissue damage in the recipient, as would be the case if blood group O thrombocyte preparations were used.

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