Expression of Blood Group A Antigens in Human Bone Marrow Cells

By Kimmo K. Karhi, Leif C. Andersson, Pekka Vuopio, and Carl G. Gahmberg

We have studied the appearance of blood group A-activity during hematopoiesis in human bone marrow cells by the use of the blood group A-specific lectin from Vicia cracca. Cells that bound the lectin were identified using antiserum against the lectin followed by rosetting with protein A-containing Staphylococcus aureus cells. Only cells of the erythroid lineage from blood group A individuals formed staphylococcal rosettes. A-activity occurred in basophilic normoblasts and later stages of erythropoiesis, whereas pronormoblasts were negative. The appearance of blood group A-activity coincided roughly with the onset of hemoglobin synthesis and slightly later than the expression of the major sialoglycoprotein of erythrocytes, glycophorin A. Glycophorin A did not, however, contain blood group A-activity when analyzed by immunoprecipitation and gel electrophoresis.

The human blood group A antigen activity is determined by the oligosaccharide N-acetyl-D-galactosamine α(1-3) [L-fucose α(1-2)] D-galactose attached to nonreducing terminals of blood group-active glycoconjugates in the erythrocyte membrane.

Several blood group A-active glycolipids have been isolated from red cells and their structures have been elucidated. Recently it has been shown that ABO-activity is also found in red cell glycoproteins, notably in band 3 and in the glycoproteins migrating on polyacrylamide gels in the 4.5 region.

Although we have much information on the nature of the ABO blood group-active glycoconjugates in mature red cells, little is known about the expression of these antigens on cells of earlier stages of hematopoietic differentiation.

In the present work we have identified blood group A antigen-expressing cells in bone marrow by using the blood group A-specific lectin from Vicia cracca. Human bone marrow cells were incubated with the lectin followed by rabbit antilectin antibodies. The cells binding lectin and antilectin antibodies were rosetted with protein A-containing staphylococci and identified in cytocentrifuge smears stained with benzidine and counter-stained with May-Grünewald-Giemsa.

We report that blood group A-activity is found only on the cells of the erythroid series. Blood group A antigen is detected on basophilic normoblasts and cells of later stages of the erythroid lineage.

MATERIALS AND METHODS

Chemicals and Enzymes

Acrylamide and N,N'-methylenebisacrylamide were obtained from Eastman Kodak, Rochester, N.Y. D-glucose was from J.T. Baker Chemicals B.V., Deventer, Holland. N-acetyl-D-galactosamine and lactoperoxidase were purchased from Sigma Chemical Co., St. Louis, Mo. Glucose oxidase was from Worthington, Freehold, N.J. Triton X-100 and sodium dodecyl sulfate were from BDH Chemicals, Poole, England. [125I] carrier-free, (100 mCi/ml) was obtained from New England Nuclear, Boston, Mass. 14C-labeled standard proteins were obtained from the Radiochemical Centre Ltd., Amersham, U.K.

From the Department of Bacteriology and Immunology, Transplantation Laboratory, and Third Department of Medicine University of Helsinki, Helsinki, Finland, and the Department of Immunology, Biomedicum, Uppsala University, Uppsala, Sweden.

Supported in part by the Academy of Finland, the Sigrid Juselius Foundation, the Finnish and Swedish Cancer Societies, Finska Läkaresällskapet, and the Association of Finnish Life Insurance Companies.

Submitted June 20, 1980; accepted September 25, 1980.

Address reprint requests to Carl G. Gahmberg, M.D., Department of Bacteriology and Immunology, University of Helsinki, Haartmaninkatu 3, SF 00290 Helsinki 29, Finland.

© 1981 by Grune & Stratton, Inc.

0006-4971(81)7501-0024 S01.00/0


147
lectin was confirmed by hemagglutination and binding experiments.7,8

Production of Antiserum Against the Blood Group A-Specific Lectin From Vicia Cracca

A rabbit was immunized subcutaneously with 1 mg of the blood group A-specific lectin from Vicia cracca in 0.5 ml NaCl/PO4 emulsified in 1 ml of Freund's adjuvant (Difco) with 2-wk intervals. Ten days after the fourth injection the rabbit was bled and the serum collected. One ml of the serum was first absorbed three times with 0.5 ml of packed erythrocytes (pooled from donors of different ABO blood groups) at 4°C for 2 hr, and subsequently twice with 0.5 ml of packed normal human leukocytes pooled from several healthy donors.

Visualization of Bone Marrow Cells Expressing Blood Group A-Activity

Bone marrow cells (30-50 x 10^6 cells) from donors of different ABO blood groups were suspended in 1.0 ml of cold RPMI-1640 medium containing 0.1% bovine serum albumin and 0.1 mM CaCl2-0.1 mM MgCl2 and 0.4 μg of Vicia cracca lectin was added. The concentration of the lectin was half of the concentration needed for agglutination of 1% suspension of A, erythrocytes when determined in two-fold serial dilutions. The cells were incubated on ice for 30 min and then washed twice with cold medium. The cells were then suspended in 0.5 ml of medium and 5 μl of the antilectin antiserum was added. After incubation for 30 min on ice, the cells were washed twice at 0°C and suspended in 0.2 ml of the same medium. Twenty microliters of the 10% staphylococcal suspension was added. After 30 min on ice the cells were washed twice by centrifugation at 600 g for 6 min to remove loose bacteria. Cell smears were prepared by use of a cytocentrifuge (Shandon Southern Products, Ltd., Cheshire, U.K.). The cells were stained with the Lephene's modification of the benzidine reaction17 to detect cells containing hemoglobin and counter-stained with May-Grünwald-Giemsa stain. Control experiments were done: (A) in the presence of 10 mM N-acetyl-D-galactosamine, (B) without the lectin, and (C) without both the lectin and the antiserum but with the staphylococci.

Immunoprecipitations and Polyacrylamide Gel Electrophoresis

Two hundred microliter-samples of the 10% staphylococcal suspension were coated with 10 μl of the antilectin antiserum and after three washes incubated with 30 μg of Vicia cracca lectin. After two washes, the staphylococci were added to samples of 125I-labeled erythrocyte membranes solubilized in 1% Triton X-100 in NaCl/PO4 and incubated on ice for 2 hr. The staphylococci were washed three times with the Triton X-100-NaCl/PO4, and the bound molecules eluted by boiling with 300 μl of 1% sodium dodecyl sulfate for 2 min. The supernatants were lyophilized and solubilized in sample buffer containing 3 M urea. Samples were electrophoresed on 8% polyacrylamide slab gels in the presence of sodium dodecyl sulfate.18 The gel was fixed with 20% sulfosalicylic acid, vacuum dried and covered with Kodak X-Omat R film, and stored at -70°C for 1 day.

RESULTS

Identification of Bone Marrow Cells Expressing Blood Group A Antigens

Rosette formation could be detected only in bone marrow cell samples from donors of blood group A.
and binding could be seen in polychromatic normoblasts. The pronormoblasts were negative for both group A antigen coincided with the presence of hemoglobin. This indicates that this binding was not blood amine. This confirms the specificity of binding (Fig. 1B).

Patients Who Had Surgery

Of the 170 patients clinically suspected of having appendicitis, 60 underwent surgery. Forty-five had pathologically proved appendicitis, including five cases with histologic evidence of chronic inflammation with superimposed acute signs of inflammation of the appendix (Fig. 3). Of these patients, appendicitis was correctly diagnosed preoperatively by sonography in 42. Histologic and sonographic correlation was done in 25 cases of appendicitis. The proximal part of the appendix was the most accurate site for measuring wall thickness, and hyperechoic fat made the submucosa a useful landmark for identifying the wall on sonography. Clear distinction of the layers of the wall allowed an accurate measurement in 21 cases. The wall thickness on sonography varied between 3.3 and 7.5 mm (mean, 4.55 mm). Corresponding values by histopathology varied between 3.5 and 7.7 mm (mean, 4.58). Thirteen cases of appendicitis had advanced wall inflammation in the distal half, which was indistinguishable on sonography from hypoechoic intraluminal pus. Similarly, the appendiceal wall was not clearly visualized in four other cases, and noncompressibility as well as findings of periappendicitis were criteria used to diagnose appendicitis.

Three studies were incorrectly interpreted as normal. One false-negative examination occurred with nonvisualization of a retrocecal appendix in an obese patient, although pain occurred with transducer compression in the right lower quadrant. Another case involved an initial interpretive error; retrospectively, the sonogram showed classic signs of appendicitis with a wall thickness of 4.5 mm and hypoechoic surrounding fat with a small amount of ascites (Fig. 4). In the third case, only the proximal half of a normal appendix was...
Fig. 2. Autoradiography of the electrophoretic patterns of 12C-labeled erythrocyte membranes and immunoprecipitates. (A) 14C-labeled standard proteins. M = myosin, PHb = phosphorylase b, BSA = bovine serum albumin, OA = ovalbumin, CA = carbonic anhydrase. (B) Pattern obtained from normal human erythrocytes labeled with 12C. (C) Pattern obtained from A erythrocytes with Vicia cracca lectin-containing staphylococci. (D) As in Fig. 2C, but in the presence of 10 mM N-acetyl-O-galactosamine. (E) Pattern obtained from A erythrocytes when preimmunization serum was used instead of antilectin antiserum. (F) Pattern obtained from B erythrocytes with Vicia cracca lectin-containing staphylococci.

and at present no good techniques for separating such cells are available.

REFERENCES


Expression of blood group A antigens in human bone marrow cells

KK Karhi, LC Andersson, P Vuopio and CG Gahmberg