Suppressed Expression of Blood Group B Antigen and Blood Group Galactosyltransferase in a Preleukemic Subject

By Akira Yoshida, Takashi Kumazaki, Vibha Davé, Joyce Blank, and Walter H. Dzik

The B antigen activity was severely diminished in a patient's RBCs at the preleukemic stage prior to chemo- or radiotherapy. The amount of H sites of the patient's RBC membranes was found to be comparable to that of O RBC membranes. The activity of α(1→2) fucosyltransferase (H enzyme) was not severely decreased in the patient's plasma and bone marrow. However, the activity of α(1→3) galactosyltransferase (B enzyme), which converts H substance to B substance, was drastically reduced in the patient's bone marrow. Thus, the diminished B antigen in the patient's RBCs was caused mainly by the blockage of conversion of the H substance to B substance. It is suggested that the viral oncogene linked to the ABO locus at q34 of chromosome No. 9 would occasionally suppress the expression of blood group A and B enzymes and A and B antigens.

Quantitative and/or qualitative disturbances of the blood group ABH antigens and glycosyltransferases involved in synthesis of the blood group antigens have been observed in various types of carcinoma.1-4 Severe suppression of the blood group ABH activity in RBCs was occasionally found in leukemic patients.5 The blood group ABH substances are produced from their common precursor by the action of specific glycosyltransferases, ie, α(1→2) fucosyltransferase (H enzyme) for synthesis of the H substance, α(1→3) N-acetylgalactosaminyl transferase (A enzyme) for synthesis of the A substance, and α(1→3) galactosyltransferase (B enzyme) for synthesis of the B substance. Therefore, suppression of the blood antigen activity in leukemic RBCs could be induced by the suppression of one of these blood group glycosyltransferases. The previous studies on the problem are controversial. We examined the amounts of H substance in RBC membranes, and H enzyme and B enzyme activities in the plasma and bone marrow of a preleukemic patient whose RBC B antigen activity was severely diminished.

Materials and Methods

Case description. The patient was a 58-year-old Caucasian male (blood type B, nonsecretor) initially diagnosed in 1979 with paroxysmal nocturnal hemoglobinuria based upon intermittent hemolysis and a positive Ham test on two separate occasions. In 1982, splenomegaly developed. In Aug 1984, he was admitted to the hospital with 30-lb weight loss, night sweats, early satiety, and left upper abdominal pain. Blood grouping showed diminished expression of group B antigen and Kidd antigens for the first time. Splenomegaly was present. The hemoglobin level was 9 g/dL. Sodium water lysis test was negative on four occasions. A bone marrow examination was consistent with preleukemia. The patient was transfused with 2 units of packed B RBCs on Sept 30, Oct 2, and Oct 14, 1984. Blood sample (with acid-citrate-dextrose [ACD] anticoagulant) was taken on Oct 21 for biochemical examination. At the end of Jan 1984, he was again admitted for left upper quadrant abdominal pain. Bone marrow (in heparin) and blood (with EDTA anticoagulant) samples were taken on Feb 2, 1984, for further examination. At that time, the patient was diagnosed as having myelomonocytic leukemia with an elevated peripheral leukocyte count and was transfused with 2 units of packed B RBCs and 5 units of washed frozen O RBCs, and he was begun on treatment with vincristine and prednisone. The patient died on the 12th hospital day from an intracranial hemorrhage. Chromosome analysis indicated some abnormalities, ie, XY, -7 in each of the 27 metaphases examined.

Blood and bone marrow. Control samples were taken from a leukemic subject, nonleukemic patients, and healthy blood and bone marrow donors, after obtaining their written consent. These samples had usual ABH activities in their RBCs. Bone marrow samples were centrifuged at 10,000 g for ten minutes, and the supernatant and the precipitate, which contained blood cells at various stages of their development, were separated. The precipitate was washed twice with 0.02 mol/L phosphate buffer, pH 7.3, containing 0.15 mol/L NaCl by centrifugation, resuspended in 2 vol of the same buffer containing Triton X-100, 0.25%, and disintegrated by freezing-thawing. The extract was used for assay of transferase activities. Plasma and bone marrow supernatant ("bone marrow fluid") were dialyzed against 0.01 mol/L Tris·Cl buffer, pH 7.0, containing 0.05 mol/L EDTA and used for assay of transferase activities.

Assay of blood group H, A, and B transferase activities. H enzyme activity was assayed by measuring the radioactive transfer from guanosine diphosphate (GDP)-[3H]-Fuc into phenyl-α-D-Gal as previously described.6,7 and as previously described.8 A and B enzymes were assayed by measuring the radioactivity transferred from UDP-[3H]-GalNAc (for A enzyme assay) or UDP-[3H]-Gal (for B enzyme assay) into 2-fucosyllactose as previously described.9 One unit of activity catalyzed the transfer of 1 mmol of the sugar to the acceptor for one hour at 37°C. Sources of the substances (ie, UDP-Gal, UDP-[3H]-Gal, UDP-GalNAc, UDP-[3H]-GalNAc, GDP-[3H]-Fuc, 2-fucosyllactose, and phenyl-α-D-Gal were described).9

Fractionation of patient's RBCs. Since the patient was transfused with 2 units of B RBCs seven days before the venesection, the blood samples contained the patient's own RBCs and donor's RBCs. In order to separate these two populations, 0.7 mL of packed RBCs were suspended in 7 mL of saline and mixed with 0.12 mL of anti-B agglutinin (1:5 dilution, Ortho Diagnostics, Raritan, NJ). After incubating the suspension for 30 minutes at 25°C, unagglutinated cells (in the upper layer) and agglutinated cells (in the bottom) were separated. Anti-B agglutinin was added to the unagglutinated RBCs, and the differential agglutination was repeated. Approximately 60% of the original RBCs remained unagglutinated after agglutination four times. More than 98% of RBCs in control B blood were agglutinated by the same procedure. The unagglutinated RBCs were considered to be the patient's RBCs. The agglutination titer of the patient's RBCs thus obtained was <32, in comparison to the control B titer of 512 (the titer was expressed as a maximum dilution of the standard B agglutinin, which caused clear agglutination after...
30 minutes' incubation at 25 °C). The patient's RBCs exhibited high H activity (agglutinability with Ulex europaeus anti-H lectin), which was comparable or slightly lower than control O RBCs.

Quantification of H sites in RBC membranes. RBC membranes, prepared by the method of Dodge, were incubated with UDP-[3H]-GalNAc and the highly purified Aβ enzyme, and the amounts of GalNAc incorporated into the membranes at saturation were determined, as previously described.

RESULTS

The B antigen activity of the patient's RBCs was severely diminished and the H antigen activity was conversely elevated. The number of H antigen sites of the patient's RBCs was 0.74 × 10^6 per cell. Since the number of H sites of control O RBCs ranged from 0.9 to 1.2 × 10^7 per cell, the amount of H sites was not substantially decreased in the patient's RBC membranes.

The H enzyme activity of the patient's plasma, bone marrow fluid (membrane bound enzyme) was somewhat lower than the average of control samples (Table 1). However, the activity of another leukemic subject with no blood group antigen abnormality was also lower than the average.

Substantial decrease in the B enzyme activity was noticeable in the patient's plasma and bone marrow fluid (Table 1). The B enzyme activity of the patient's bone marrow blood cells (membrane bound enzyme) was too low to be detected. The H enzyme activity of the patient's bone marrow blood cells was 0.006 U/mL, and that of control samples ranged from 0.010 to 0.022 U/mL.

DISCUSSION

Suppressed blood group ABH activity in RBCs and other tissues could result from a decreased activity of glycosyltransferases involved in the synthesis of the blood group substances. Watkins et al reported that plasma H enzyme activity, measured using phenyl-β-D-Gal as an acceptor, was substantially lower in leukemic patients without chemotherapy than in control subjects. By contrast, Khilamani et al and Bauer et al reported that plasma α(1→2) Fuc-transf erase (corresponding to H enzyme) activity, measured using desialofetuin as an acceptor, and α(1→3) Fuc-transferase, measured using desialodegallactofetuin as an acceptor, were elevated in leukemia and colon carcinomas and returned to the normal level in remission. No rational explanation has been presented for the totally opposite observations, i.e., a diminution of the H enzyme found by one laboratory and an elevation of the H enzyme observed by other laboratories. In a leukemic patient with diminished RBC A antigen, morphological changes were observed, but A enzyme activity was found to be unimpaired. An acquired diminution in the expression of A, H, M, i, and I antigens was observed in a patient with lymphocytic lymphoma. No significant decrease of H and A enzyme activities was detected in the patient's serum, but marked augmentation of the A and i antigens was observed on the patient's RBCs by neuraminidase treatment.

Hakomori et al observed a depletion of blood group A and B activities in cancerous tissues, and reported that the activities of A and B enzymes in gastrointestinal epithelium in cancerous tissues were only one fifth to one sixth of those in noncancerous tissues of the same individuals.

Only about 25% of plasma A and B enzymes originate from hematopoietic tissues. In contrast, plasma H enzyme was assumed to be solely derived from bone marrow. This assumption is questionable, since there is no evidence that the expression of the H gene is restricted in hematopoietic tissues. H enzyme activity in bone marrow fluid of the patient (nonsecretor) was found to be equal or often lower than in plasma (Table 1), suggesting that part of the plasma H enzyme was derived from tissues other than bone marrow. In order to define a change of blood group transferases in leukemia and other carcinomas, the enzyme activities of bone marrow and affected tissues should be examined, as was done by Steller and Hakomori.

In the present preleukemic patient with diminished RBC B antigen activity, the amount of H sites in his RBC membranes was near normal, and activity of H enzyme in his plasma and bone marrow was not markedly decreased. On the other hand, the activity of B enzyme was found to be severely diminished in the patient's bone marrow fluid and bone marrow blood cells. Therefore, the major cause of the diminished RBC B antigen activity in the present case is likely due to the blockage of conversion of the H substance to the B substance, not due to the decrease in synthesis of the H substance.

Based on the observation of independent expression of A- and B-like antigen activities in gastric carcinoma of AB individuals, Denk et al suggested the possibility of deletion or repression of either A or B gene in the ABO locus in given cancerous tissues. Salmon et al observed the activity of adenylyl kinase (AK) in RBCs with diminished A antigen activity, and suggested linkage between the ABO and AK loci.

Recent studies indicated that the human viral oncogene c-abl is located at q34 position of chromosome No. 9. Chromosomal abnormalities, i.e., translocation involving chromosomes No. 9 and 22, were observed in leukemia in a high frequency. The blood group ABO locus is in q34 of chromosome No. 9. It is conceivable that the viral oncogene may occasionally disturb the expression of ABO locus resulting in depletion of blood group A and B activities in RBCs of leukemic patients.

ACKNOWLEDGMENT

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Table 1. Blood Groups A, B, and H Enzyme Activities

<table>
<thead>
<tr>
<th>Patient (Preleukemia)</th>
<th>B</th>
<th>A Enzyme</th>
<th>B Enzyme</th>
<th>H Enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Plasma</td>
<td>Bone Marrow Fluid</td>
<td>Plasma</td>
</tr>
<tr>
<td>1 (Leukemia)</td>
<td>B</td>
<td>0.088</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>2 (ludopathic</td>
<td>B</td>
<td>0.07</td>
<td>0.132</td>
<td>0.018</td>
</tr>
<tr>
<td>thrombocytopenic purpura)</td>
<td>0.018</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 (Sideroblastic anemia)</td>
<td>A</td>
<td>0.34</td>
<td>0.23</td>
<td>0.03</td>
</tr>
<tr>
<td>4 (bone marrow donor)</td>
<td>A</td>
<td>0.72</td>
<td>0.22</td>
<td>0.03</td>
</tr>
<tr>
<td>5 (bone marrow donor)</td>
<td>A</td>
<td>(0.25)</td>
<td>(0.18)</td>
<td></td>
</tr>
<tr>
<td>6 (bone marrow donor)</td>
<td>A</td>
<td>(0.13)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control A</td>
<td>A</td>
<td>0.72</td>
<td>0.36</td>
<td></td>
</tr>
<tr>
<td>Control A</td>
<td>A</td>
<td>0.44</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>Control B</td>
<td>B</td>
<td>0.26</td>
<td>0.028</td>
<td></td>
</tr>
<tr>
<td>Control B</td>
<td>B</td>
<td>0.22</td>
<td>0.025</td>
<td></td>
</tr>
</tbody>
</table>

The patient's samples were taken and examined on two occasions. A enzyme activity was assayed at its optimum pH at 6.0 in the case of A, and at its optimum pH at 8.0 in the case of A2. The values given in parentheses are the activity levels at pH 8.0. B enzyme activity was assayed at pH 7.0. The values are expressed in enzyme units per milliliter of plasma or bone marrow fluid. Subjects No. 4, 5, and 6 are healthy bone marrow donors, and all control subjects are healthy blood donors.

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