von Willebrand factor (vWF) is synthesized exclusively by endothelial cells and megakaryocytes, and stored in the intracellular granules or constitutively secreted into plasma. ABO blood group antigens are covalently associated with asparaginyl-linked sugar chains of plasma vWF. The effect of ABO-mismatched bone marrow transplantation (BMT) or blood stem cell transplantation (BSCT) on the expression of ABO blood group antigens on the vWF was examined to obtain information on the origin of these antigens. In ABO-mismatched (HLA-matched) groups, 8 cases of BMT and 4 cases of BSCT were examined. In all cases, the ABO blood groups on red blood cells were gradually converted to the donor's type within 80 to 90 days after the transplantation. The blood group antigens on the vWF were consistent with the recipient's blood group for the period monitored by enzyme-linked immunosorbent assay (ELISA). When vWF was isolated from normal platelets and examined for the blood group antigens using ELISA or immunoblotting, it showed few antigens. However, vWF extracted from veins expressed blood group antigens. These findings indicate that platelet (megakaryocyte)-derived vWF does not contain blood group antigens and that these antigens may be specifically associated with vWF synthesized in endothelial cells and secreted into plasma. Furthermore, it is possible that the persistence of the recipient's blood group antigens on plasma glycoproteins such as vWF, independent of the donor-derived erythrocytes, after ABO-mismatched stem cell transplantation, may influence the immunological system in the production of anti-blood group antibodies resulting in the establishment of immunological tolerance in the recipient plasma.

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MATERIALS AND METHODS

Materials. Plasma samples were collected from normal adults, and from donors and recipients of ABO-matched (three cases) or mismatched BMT (four cases of minor, three cases of major, and one case of major-minor mismatched, but HLA-matched) at intervals before and after transplantation, and stored at −80°C after addition of 1/50 volume of 4 mmol/L EDTA, 4 mmol/L N-ethylmaleimide, 4 mmol/L benzamidine, 400 kallikrein inhibitor units/mL of aprotinin. Three cases of allogeneic peripheral blood and one case of cord blood stem cell transplantation were also examined. Standard vWF was purified from FVIII concentrates as described previously.22

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Enzyme-linked immunosorbent assay (ELISA). ELISA plate (Immu-no module; Nunc Intermed, Kamstrup, Denmark) was coated with 50 or 100 µL (in each well) of anti-vWF goat immunoglobulin G (IgG) (20 µg/mL; Medical and Biological Laboratory (MBL), Nagoya, Japan) in 100 mmol/L bicarbonate buffer, pH 9.6 overnight at 4°C, and blocked with 200 µL of Tris-buffered saline (TBS; 10 mmol/L Tris-HCl, pH 7.5, 150 mmol/L NaCl) containing 1% bovine serum albumin (BSA) overnight at 4°C. The coated plate was used within 3 weeks after preparation and washed twice with 200 µL of TBS containing 0.05% tween 20 (TwTBS) before use. Plasma samples were appropriately diluted (10 to 100-fold with TwTBS) and 50 or 100 µL of each was applied to the anti-vWF coated plate and incubated for 90 minutes at room temperature. The plate was washed five times with 200 µL of TwTBS and incubated with either 50 or 100 µL of anti-A, anti-B monoclonal antibody (MoAb) (Ortho Diagnostics Systems, Raritan, NJ) diluted with TwTBS (10-fold for anti-A, fivefold for anti-B), biotin-conjugated UEA-I lectin (5 µg/mL, EY Laboratories, San Mateo, CA) that recognizes the type-2 H structure rich in blood group O, or horseradish peroxidase (HRP)-conjugated anti-vWF rabbit IgG (1/1,000 diluted with TwTBS, Zymed Laboratories, San Francisco, CA) and vWF in the vein extracts and normal plasma were measured by sandwich ELISA using the corresponding HRP-conjugated antibodies (MBL, Dakopatts).

Other methods. The blood group of the red blood cells and the anti-blood group antibodies was determined by conventional hemagglutination assay using anti-A and -B MoAbs and standard human type A1 and B red blood cells (Ortho Diagnostics) according to the manufacturer’s instructions.

RESULTS

ABO blood group antigens on red blood cells. ABO blood group antigens on the surface of red blood cells and the anti-blood group antibodies in the plasma of the recipient with acute lymphocytic leukemia in first relapse (blood group AB, male, 36 years old), who was transplanted from a sibling donor (blood group O, female, 35 years old), were monitored for 161 days at intervals before and after BMT (Fig 1A) (the recipient experienced complete remission, but relapsed 7 months after transplantation and died of fungal infection even though a second BMT was performed). The recipient’s red blood cells with type AB showed a mixed field with anti-A and -B antibodies 1 week after transplantation, but had completely lost their reactivity to the antibodies after 90 days indicating that the blood group of the recipient was gradually converted to the donor’s type by ABO-mismatched BMT. The anti-blood group antibodies assayed by standard red blood cells (type A1 and B) showed that there was no production of either of these antibodies for the duration of the monitoring period (130 days after transplantation) (Fig 1A). In all cases of ABO-mismatched BMT examined, the antibodies against the recipient’s original blood group were not found in the plasma (Table 1).

ABO blood group antigens on plasma vWF. vWF in plasma was monitored using ELISA. Concentration of the vWF in the patients receiving a BMT was significantly higher (204 ± 42 U/dL, n = 6, P < .001) when compared with the average concentration in normal adults (91 ± 22 U/dL, n = 9) regardless of ABO-matched or mismatched BMT (Table 1). Plasma vWF, especially, was transiently increased after transplantation and gradually decreased as reported25,26 (Fig 1A and 1B). The level of ABO blood group antigens on the vWF also varied with the concentration of vWF, but it never converted to the donor’s type after ABO-mismatched BMT (Fig 1A and 1B). UEA-I lectin reacts with vWF from blood group O because it has more H-substance than the other groups.2 Reactivity of the recipient’s plasma vWF against UEA-I was less than that of the donor’s vWF except for a short period after BMT (Fig 1A).

In the case of major mismatched BMT from a type A donor to a type O recipient (Fig 2), plasma vWF of the recipient at 7 months after transplantation still showed UEA-I binding activity similar to the level before transplantation. No reactivity was shown to anti-A antibody, indicating neither a significant reduction of the vWF with blood group O, nor the production of vWF with blood group A antigen.

In the eight cases of ABO-mismatched BMT examined, there

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was no change in the blood group of plasma vWF (Table 1). Examination 3 years, 9 months after transplantation showed that the vWF still continued to express the recipient’s blood group antigens but not the donor’s type.

In addition to bone marrow, BSCT was also examined. Three cases of allogeneic peripheral and one case of cord ABO-mismatched BSCT showed the same results as BMT (Table 2). Plasma vWF expressed the original blood groups in contrast to red blood cells, which were converted to express the donor’s type.

**Blood group antigens on vWF from platelets and vein.** To elucidate the origin of blood group antigens on plasma vWF, we examined vWF extracted from both platelets and the renal vein. vWF immunoprecipitated from platelets and plasma both showed the 270 kD subunit band with some minor degraded bands by immunoblotting with anti-vWF antibody (Fig 3). Plasma vWF showed the corresponding blood group, whereas platelet vWF had no or only a faint blood group antigenicity. The latter showed two bands at about 110 and 130 kD that were reactive to the corresponding anti-blood group antibody, but did not react with anti-vWF antibody. Also using ELISA, platelet vWF showed no significant binding to the anti-blood group antibody (Fig 4).

vWF in the renal vein extracts was examined using ELISA. Among the samples from two subjects with type A and one subject with type O, vWF from the type A subjects clearly showed blood group A antigenicity, although the reactivity was about half that of plasma vWF from normal blood type A subjects (Fig 4). Vein vWF from the type O subject showed only UEA-I reactivity (data not shown). To address the question of whether vWF in the vein extracts used was mostly derived from contaminated plasma, we measured the contents of several plasma proteins in the extracts and compared them with those in normal plasma. Fibrinogen, transferrin, and α₂-macroglobulin in the vein extracts used were estimated to be 0.4 ± 0.1, 0.4 ± 0.1, and 0.6 ± 0.3 U/dL, respectively (n = 3), whereas the vWF in the extracts was 12.2 ± 2.2 U/dL. These findings suggest that the vein extracts contained a greater amount of vWF compared with other plasma proteins and that vWF derived from plasma appeared to be less than 1%.

**DISCUSSION**

HLA-matched but ABO-mismatched BMT has no influence on marrow engraftment, graft rejection, and graft-versus-host disease if appropriate care, such as plasma exchange and antibody absorption, is performed to avoid acute hemolysis. However, delayed hemolysis, retarded growth of erythroblast, or undergrown erythrocytes have often been observed as complications. Production of anti-blood group antibody must be controlled by the remaining host antigens and the donor-derived lymphocytes. It has been reported that blood group antigens are covalently linked to vWF, FVIII, and other glycoproteins such as vWF might contribute to the establishment of immunological tolerance.

The finding that plasma vWF continued to express the recipient’s blood group after ABO-incompatible BMT (and BSCT). No anti-blood group antibody against the recipient’s original blood group was detected after the transplantation. Wernet and Mayer reported that isoagglutinins against the recipient’s original red blood cell type are produced only during the early days after transplantation even though the patient has converted to the donor’s red blood cell type after ABO-mismatched BMT. Although it is probable that the immunosuppressing treatment might interfere with the production of antibodies in the recipient, the observed pattern of antibody production (Table 1) suggests that the remaining blood group antigens on plasma glycoproteins such as vWF might contribute to the establishment of immunological tolerance.

The finding that plasma vWF continued to express the recipient’s blood group after ABO-mismatched BMT suggested two possibilities for the origin of these antigens on the vWF. One was that vWF produced in megakaryocytes, differentiated from bone marrow stem cells, would not be secreted but stored in platelets. The other was that vWF in platelets originally had no blood group antigens. Almost all plasma vWF has been shown to be supplied from endothelial cells rather than platelets.

![Image](https://www.bloodjournal.org/issue-files/2897.jpg)
by crossed BMT of pigs, suggesting that the secretion of vWF from platelets is limited to the local area at thrombosis. Platelets have been known to have both covalently and noncovalently bound blood group antigens. Recently, the covalently bound antigens have been found in platelet membrane glycoproteins (GP) such as GPIa, Ib, Iia, IIb, IIIa, IV, and V, suggesting that platelets (megakaryocytes) have a machinery to assemble the blood group antigens. We have prepared platelet vWF, but it has no or only a faint blood group antigenicity. The 110 and 130 kD proteins observed in the immunoprecipitated platelet vWF (Fig 3) seem to be GPs coprecipitated with vWF. The absence of blood group antigens in platelet vWF has also been recently reported by two groups. The very faint blood group reactivity observed in the platelet vWF (Fig 3 and 4) might be a contamination from the plasma vWF adsorbed onto the platelets.

Another vWF producing site is endothelial cells. We found that the renal vein extracts contained vWF with blood group antigens. Expression of the blood group antigens by the vWF molecule was about half that of the plasma vWF, suggesting that vWF molecules with no or a small amount of blood group antigens also exist. It is possible that these vWFs are incompletely glycosylated. Alternatively, the glycosylation may be different between vWF that is constitutively secreted and that stored in the regulated Weibel-Palade body pathway. Recently, Yamamoto et al reported that the synthesis of vWF in endothelial cells varied among the organs in mice. Glycosylation is regulated by glycosyltransferases and trimming glycosidases in cells, suggesting that the blood group antigen production might also be altered by each organ. When we analyzed vWF extracted from cultured human umbilical vein endothelial cells, no significant blood group antigens were observed and neonatal plasma vWF showed a lower expression of these antigens (Matsui, unpublished observations), suggesting that the blood group antigens on plasma glycoproteins may also be developmentally regulated like embryonic antigens.

Our present findings, together with the recent findings of Brown et al showing that plasma vWF with blood group antigens was rapidly increased after administration of DDAVP to a type I von Willebrand disease patient, strongly suggest that vWF with blood group antigens is specifically glycosylated in endothelial cells but not in megakaryocytes. It is also possible that the blood group antigens are attached to vWF extracellularly by plasma glycosyltransferases after secretion. A or B

<table>
<thead>
<tr>
<th>Case</th>
<th>Recipient Sex (age)</th>
<th>Diagnosis</th>
<th>Blood Group Recipient</th>
<th>Time after BMT (months)</th>
<th>vWF Conc. (U/mL)</th>
<th>Blood Group after BMT</th>
<th>Anti-blood Group Antibody Production</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABO-Mismatched BMT Major</td>
<td>M.H.</td>
<td>M (39)</td>
<td>CML</td>
<td>B</td>
<td>AB</td>
<td>11</td>
<td>171</td>
</tr>
<tr>
<td></td>
<td>S.Y.</td>
<td>F (9)</td>
<td>ALL</td>
<td>O</td>
<td>A</td>
<td>7</td>
<td>169</td>
</tr>
<tr>
<td>Minor</td>
<td>K.Y.</td>
<td>M (17)</td>
<td>SAA</td>
<td>A</td>
<td>O</td>
<td>9</td>
<td>144</td>
</tr>
<tr>
<td></td>
<td>T.T.</td>
<td>M (13)</td>
<td>WAS</td>
<td>B</td>
<td>O</td>
<td>45</td>
<td>163</td>
</tr>
<tr>
<td></td>
<td>M.O.</td>
<td>F (24)</td>
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<td>B</td>
<td>O</td>
<td>10</td>
<td>92</td>
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<td>O</td>
<td>5</td>
<td>278</td>
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<tr>
<td>Major + Minor</td>
<td>K.F.</td>
<td>F (12)</td>
<td>PRCA</td>
<td>A</td>
<td>B</td>
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<td>234</td>
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<tr>
<td>ABO-Matched BMT</td>
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<td>MDS</td>
<td>A</td>
<td>A</td>
<td>18</td>
<td>189</td>
</tr>
<tr>
<td></td>
<td>T.H.</td>
<td>M (37)</td>
<td>ALL</td>
<td>B</td>
<td>B</td>
<td>35</td>
<td>339</td>
</tr>
<tr>
<td></td>
<td>M.I.</td>
<td>F (24)</td>
<td>AML</td>
<td>AB</td>
<td>AB</td>
<td>6</td>
<td>272</td>
</tr>
</tbody>
</table>

CML: chronic myeloid leukemia; ALL: acute lymphocytic leukemia; AML: acute myelogenous leukemia; SAA: severe aplastic anemia; WAS: Wiskott-Aldrich syndrome; MDS: myelodysplastic syndrome; PRCA: pure red cell aplasia; n.d.: not determined.
transferases are still present in plasma in accordance with the recipient's type even after ABO-mismatched BMT. However, transplanted O-type erythrocytes to type-A recipients did not show A antigens even though the plasma contained A-type vWF and A transferase (Sako, unpublished observation). Furthermore, it is not likely that the plasma contains enough sugar nucleotide donors such as UDP-N-acetylgalactosamine and UDP-galactose for A and B transferase, respectively.

Although the biological function of the blood group antigens on vWF is still not clear, the different glycosylation between platelet and endothelial vWF might influence the function of each pool of vWF in hemostasis or in its association with FVIII. Recently, Sarode et al reported that the blood group sugar chains on vWF influenced the ristocetin-induced platelet agglutinating activity. Further studies on the relationships between thrombotic complications followed by ABO-mismatched BMT or BSCT and the presence of blood group antigens on vWF may contribute to more successful transplantation.

Table 2. Blood Group Antigens on Plasma vWF and RBC of the Recipients after ABO-Mismatched Allogeneic-Peripheral Blood Stem Cell Transplantation (PBSCT) and Cord Blood Stem Cell Transplantation (CBT)

<table>
<thead>
<tr>
<th>Case</th>
<th>Recipient</th>
<th>Sex (age)</th>
<th>Diagnosis</th>
<th>Blood Group</th>
<th>Time after Transplantation (months)</th>
<th>vWF Conc. (U/dL)</th>
<th>Blood Group after Transplantation</th>
<th>Anti-blood Group Antibody Production</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBSCT</td>
<td>N.T.</td>
<td>M (16)</td>
<td>CML</td>
<td>A</td>
<td>7</td>
<td>247</td>
<td>A</td>
<td>O</td>
</tr>
<tr>
<td></td>
<td>A.T.</td>
<td>M (3)</td>
<td>JMML</td>
<td>O</td>
<td>21</td>
<td>169</td>
<td>O</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>M.I.</td>
<td>F (8)</td>
<td>ALL</td>
<td>O</td>
<td>15</td>
<td>117</td>
<td>O</td>
<td>A</td>
</tr>
<tr>
<td>CBT</td>
<td>S.Y.</td>
<td>F (3)</td>
<td>FHL</td>
<td>B</td>
<td>6</td>
<td>202</td>
<td>B</td>
<td>O</td>
</tr>
</tbody>
</table>

JMML: juvenile myelomonocytic leukemia; FHL: familial hemophagocytic lymphohistiocytosis.

Fig 3. Blood group antigens on vWF from plasma and platelets. vWF was immunoprecipitated from plasma (Pls) and the platelets (Plt) extracts from normal subjects with blood groups A and B. Aliquots of each immunoprecipitates and the standard vWF (vWF, 0.5 μg) were solubilized in SDS-buffer and subjected to SDS-PAGE under reducing conditions. Proteins were transferred to a PVDF membrane followed by immunoblotting with anti-vWF antibody, anti-A and B MoAbs. vWF showed the subunit band at about 270 kD. Blood group antigens were detected on the vWF band including minor degraded band at about 140 kD prepared from plasma and the purified vWF. vWF from platelets showed no or a very faint reactivity against blood group antibody, but platelets contained smaller bands at about 110 and 130 kD that weakly reacted with anti-blood group antibodies but not with anti-vWF antibody. Numbers on the left indicate the positions of molecular mass standard (kDa). The same results were obtained when using the other two platelet specimens from blood groups A and B.

Fig 4. Blood group antigens on vWF extracted from renal veins and platelets. vWFs in the normal plasma (Pls) and in the extracts of veins and platelets (Plt) from the subjects with type A were captured with anti-vWF antibody on an ELISA plate. The concentration of vWF and the reactivity against anti-A MoAb was measured and normalized as the anti-A reactivity (A490) of 6 U/dL (1 μg/mL) of vWF solution. Each value indicates means ± SD (n = 3 except for type A vein, n = 2).
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ABO Blood Group Antigens on Human Plasma von Willebrand Factor After ABO-Mismatched Bone Marrow Transplantation

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