Disseminated Intravascular Coagulation in Transfusion-associated Graft-versus-Host Disease

Report of Two Cases*

MASAHIRO MURAKAWA, M.D.,†
TAKASHI OKAMURA, M.D.,† MINE HARADA, M.D.,‡
TAITO ESAKI, M.D.,† TAKUMI KAMURA, M.D.,†
TSUNEFUMI SHIBUYA, M.D.,§
and YOSHIYUKI NIHO, M.D.†

†First Department of Internal Medicine, Faculty of Medicine, Kyushu University, Fukuoka, Japan
and
‡Second Department of Internal Medicine, School of Medicine, Okayama University, Okayama, Japan
and
§Department of Internal Medicine, Hamanomachi Hospital, Fukuoka, Japan

ABSTRACT

In two cases of transfusion-associated graft-versus-host disease (TA-GVHD), severe bleeding tendencies were observed besides the characteristic features of the disease. An elevation of fibrin degradation products (FDP), D-dimer, thrombin-antithrombin III complex (TAT) and plasmin-α₂ plasmin inhibitor complex (PIC), was revealed. These findings were compatible with disseminated intravascular coagulation (DIC). To our knowledge, the association of TA-GVHD and DIC has not been described, and it is our speculation that allogeneic immune reactions may play some pathogenic role in developing DIC in TA-GVHD. The DIC may be responsible for the rapid progression of multiple organ failure (MOF) in TA-GVHD.

Introduction

It is well recognized that graft-versus-host (GVH) reaction occurs in allogeneic bone marrow transplantation (BMT), in which T-lymphocytes in the graft mount cytotoxic reactions against host tissues. Blood transfusion, which is a kind of organ transplantation, has been found to induce GVH reactions in certain situations.1,2,3 This disorder, known as transfusion-associated graft-versus-host dis-
ease (TA-GVHD), is characterized by a rapid progression leading to high mortality as compared to GVHD in allogeneic BMT. The TA-GVHD is usually associated with pancytopenia in addition to common GVHD lesions such as erythroderma, liver damage, and diarrhea. The majority of TA-GVHD does not respond to any intensive therapies, and the primary cause of death in most patients is multiple organ failure (MOF). Although thrombocytopenia, as well as leukocytopenia, is common in TA-GVHD, hemorrhagic manifestations have not been fully investigated.

Two patients with TA-GVHD have been confirmed by histological findings of the skin and analysis of human leukocyte antigen (HLA). Both instances were complicated by severe bleeding tendencies besides the characteristic features of TA-GVHD. Laboratory findings in both cases revealed the presence of disseminated intravascular coagulation (DIC). To our knowledge, the association of TA-GVHD and DIC has not been described, although DIC in association with blood transfusions is known to occur in cases with hemolytic transfusion reactions such as incompatible transfusion and massive transfusion. In the present paper, two cases of DIC in association with TA-GVHD are described and a possible mechanism is speculated.

Case Reports

Patient 1

This patient, a 58-year-old male, has been previously described on the HLA analysis by Aso et al. During open heart surgery, the patient received 1 unit of unirradiated fresh whole blood from his son. On the 16th post operational day, the patient was referred to us.

On admission, marked and progressive pancytopenia was observed besides jaundice and generalized macropapular skin rash. Oozing from the site of venous injection was also observed. Stool occult blood was strongly positive. On the diagnosis of DIC, the patient received a continuous infusion of a synthetic protease inhibitor, nafamostat mesilate at a dose of 0.2 mg/kg/h. The bleeding tendency subsided transiently, but persisted thereafter. Microbiological cultures of blood, urine and stool were negative. The patient deteriorated progressively and died of respiratory failure on the 18th day after the transfusion.

Patient 2

A 70-year-old male, who had suffered from a gastric ulcer, underwent partial gastrectomy. During the operation, he received 10 units of packed red cells and 10 units of unirradiated fresh whole blood from unrelated random donors. On the 9th day after the operation, high fever (39°C) and skin erythema developed. Broad-spectrum antibiotics were given without clinical improvement. Since drug allergy was highly suspected, he was given a small dose of oral prednisolone. On the 19th day, he was referred to us because of progressive pancytopenia and respiratory failure.

On admission, generalized macropapular skin rash, ecchymoses and persistent bleeding from gingiva and oral mucosa were seen. Peripheral blood counts were as follows; Hb 11.2 g/dl, RBC 3.75 x 10^12/l, hematocrit 32.4%, platelets 30 x 10^9/l, WBC 0.07 x 10^9/l with 4% monocytes, 88% lymphocytes and 8% atypical lymphocytes. Blood chemistry showed as follows; total bilirubin 0.9 mg/dl, aspartate aminotransferase (AST) 37 IU/l, alanine aminotransferase (ALT) 52 IU/l, lactate dehydrogenase (LDH) 710 IU/l, blood urea nitrogen 30 mg/dl, and creatinine 1.8 mg/dl. Serum C-reactive protein (CRP) was 22.0 mg/dl. Occult blood of stool and urine were strongly positive. From the histological findings of a biopsied specimen, cutaneous acute GVHD was diagnosed.

For the treatment of DIC, gabexate mesilate was started by continuous infusion at a dose of 2.0 mg/kg/h, which resulted in no improvement of bleeding tendency. A high-dose methylprednisolone therapy (20 mg/kg/d) was administered without clinical effect. Cyclosporine A (CsA) was also administered by continuous infusion at a dose of 2.0 mg/kg/d in attempt to treat the acute GVHD. However, the patient died of MOF on the 21st day after transfusion.

Laboratory Methods

HEMOSTATIC STUDIES

Fibrin degradation products (FDP) and soluble fibrin monomer complex (SFMC) were measured by the latex agglutination method and hemagglutination method, respectively. Activities of plasminogen and antithrombin III (AT III) were measured by the chromogenic substrate
method.\textsuperscript{12,13} Antigen levels of \( \alpha_2 \)-plasmin inhibitor (\( \alpha_2 \)-PI) and AT III were determined with single radial immunodiffusion (SRID). Protein C activity was measured by the activated partial thromboplastin time (APTT) method.\textsuperscript{14} D-dimer, plasmin-\( \alpha_2 \)-PI-plasmin complex (PIC), thrombin-antithrombin III complex (TAT) and type-1 plasminogen activator inhibitor (PAI-1) antigen were determined by enzyme-linked immunosorbent assay (ELISA).\textsuperscript{15,16,17,18} Von Willebrand factor antigen (vWf:Ag) was determined with Laurell’s technique.\textsuperscript{19} Factor XIII activity was measured by the dansylcadaverine method.\textsuperscript{20} All assays were carried out in duplicate and the results expressed as the mean value.

**ASSAY OF CYTOKINES**

Tumor necrosis factor-\( \alpha \) (TNF-\( \alpha \)) was measured with an IRMA kit.\textsuperscript{*} Interleukin-1\( \alpha \) (IL-1\( \alpha \)) and -1\( \beta \) (IL-1\( \beta \)) were assayed with a Human Interleukin-1\( \alpha \) ELISA kit and a Human Interleukin-1\( \beta \) ELISA kit,\textsuperscript{†} respectively. \( \gamma \)-Interferon (\( \gamma \)-IFN) was measured with radioimmuno assay by using a Human \( \gamma \)-Interferon RIA kit.\textsuperscript{‡} All assays were performed in duplicate and their mean values are given.

**Results**

**HLA STUDIES**

As previously described,\textsuperscript{7} the results of HLA studies on Patient 1 and his family members were consistent with TA-GVHD: the HLA phenotype of Patient 1 after the occurrence of GVHD was replaced by that of his son, a donor of fresh whole blood.

In Patient 2, circulating lymphocytes from the patient, his sons, and his wife were studied on the day of admission. Although the unrelated donors’ lymphocytes were not available for testing, an expected HLA phenotype of Patient 2 could be deduced from HLA typing of his family members. The HLA phenotype of Patient 2 on admission shared A24, A26, Bw61 and Bw6 antigens with the expected HLA phenotype of his own but lacked Bw54 antigen (table I). Presence of Cw10 antigen on the circulating lymphocytes of Patient 2, which was not included in the expected phenotype, indicated engraftment of the unrelated donor-derived lymphocytes. These findings suggest that the unrelated-donor derived T-cells could recognize histocompatibility antigens of Patient 2, and that the recipient’s lymphocytes failed to recognize the donor-derived lymphocytes. Thus, TA-GVHD was induced in Patient 2 while graft rejection did not occur.

**HEMOSTATIC STUDIES**

In table II are shown the data of hemostatic studies from the two patients. These data demonstrate the presence of DIC based on the marked elevation of FDP, TAT, PIC and D-dimer, and positivity for SFMC. Moreover, the plasma PAI-1 and vWf:Ag levels were markedly increased in both patients. Regrettably, serial determination of these parameters could not be performed along with the treatment.

**CYTOKINES**

As shown in table III, tumor necrosis factor-\( \alpha \) (TNF-\( \alpha \)) showed a marked elevation in both patients. The IL-1\( \alpha \) was increased in Patient 2 but not in Patient 1. The IL-1\( \beta \) levels were not affected in either patient. Levels of \( \gamma \)-IFN were also elevated in both patients.
### TABLE I

#### Human Leukocyte Antigen Studies on Patient 2

<table>
<thead>
<tr>
<th>Individual</th>
<th>HLA Antigens of the Circulating Lymphocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 2 on admission</td>
<td>A24, A26, Bw61, Bw6, Cw10</td>
</tr>
<tr>
<td>Wife</td>
<td>A11, A26, B35, Bw60, Bw6, Cw10, (Cw7)*</td>
</tr>
<tr>
<td>Son 1</td>
<td>A11, A26, B35, Bw60, Bw6, Cw9</td>
</tr>
<tr>
<td>Son 2</td>
<td>A24, A26, Bw54, Bw60, Bw6, Cw1, (Cw7)*</td>
</tr>
<tr>
<td>Son 3</td>
<td>A24, A26, Bw54, Bw60, Bw6, Cw1, (Cw7)*</td>
</tr>
<tr>
<td>Expected HLA phenotype of Patient 2</td>
<td>A24, A26, Bw54, Bw61, Bw6, Cw1, Cw9</td>
</tr>
</tbody>
</table>

Human leukocyte antigen (HLA) typing was performed by the standard NIH microcytotoxicity method. Circulating lymphocytes from Patient 2, his sons, and his wife were studied on the day of admission; however, studies on the unrelated donors were not performed. As for Patient 1, see data published by Aso T, Asano Y, Harada M, Kudo J, Fujimoto K, Okamura T, Tsuda Y, Niho Y. Acta Haematol Jpn 1989;52:1064–71.

* Weak positive reaction.

**HISTOPATHOLOGIC EXAMINATION**

Histological findings in Patient 1 supported TA-GVHD as previously reported. In this case, multiple fibrin thrombi were found in the spleen, bone marrow, renal glomeruli, ascending colon, and lymph nodes.

In Patient 2, histologic examination of the skin revealed hyperkeratosis, epidermal lymphocytic infiltration, focal liquefactive degeneration of the basal layer, and eosinophilic degeneration of keratinocytes, being compatible with TA-GVHD. At autopsy, massive hemorrhage in the intestine was observed. Bone marrow was markedly hypoplastic with an increase of histiocytic cells, some of which showed erythrophagocytosis. In the renal glomeruli, fibrin thrombi were detected by phosphotungstic acid hematoxylin (PTAH) stain.

**Discussion**

On the basis of the clinical manifestations, progressive pancytopenia, and histologic findings of the skin biopsies, diagnoses of TA-GVHD were made. These were confirmed by HLA analyses of the patients and all family members. In Patient 2, TA-GVHD appears to be caused by transfusion from the unrelated donor.

Disseminated intravascular coagulation in the two patients was manifested by persisted bleeding tendencies such as ecchymoses, gingival bleeding, marked gastrointestinal bleeding and oozing from the injection site. The laboratory data, as well as the fibrin thrombi observed in postmortem examination, were consistent with intravascular coagulation and secondary fibrinolysis. To our knowledge, DIC has not been described in association with GVHD, and the mechanism of the development of DIC in TA-GVHD is speculative.

In allogeneic immune reactions, several findings of the intravascular clotting and/or vascular endothelial cell damage have been reported: (1) in human allograft rejection, microvascular thrombosis and perivascular fibrin deposits are commonly observed; (2) allogeneic stimulation in mixed lymphocyte culture induces tissue factor type procoagulant activity on monocytes; (3) an increase of plasma vWF:Ag and serum FDP may
TABLE II
Hemostatic Studies

<table>
<thead>
<tr>
<th>Tests</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Normal or Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bleeding time</td>
<td>3.5</td>
<td>N.E.*</td>
<td>&lt; 5.0 (min)</td>
</tr>
<tr>
<td>Platelet count</td>
<td>124.0 x 10⁹</td>
<td>30.0 x 10⁹</td>
<td>&gt; 100.0 x 10⁹ (l)</td>
</tr>
<tr>
<td>Prothrombin time</td>
<td>16.1</td>
<td>11.7</td>
<td>12.1 (sec)</td>
</tr>
<tr>
<td>Activated partial</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>thromboplastin time</td>
<td>48.4</td>
<td>41.6</td>
<td>29.0 (sec)</td>
</tr>
<tr>
<td>Hepaplastin test</td>
<td>34.0</td>
<td>60.2</td>
<td>60.0 - 120.0 (%)</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>233.0</td>
<td>248.0</td>
<td>150.0 - 400.0 (mg/dl)</td>
</tr>
<tr>
<td>Fibrin degradation product</td>
<td>40.0 ≤ &lt; 80.0</td>
<td>20.0 ≤ &lt; 40.0</td>
<td>&lt; 5.0 (µg/ml)</td>
</tr>
<tr>
<td>D-dimer</td>
<td>7,690.0</td>
<td>1,260.0</td>
<td>&lt; 150.0 (ng/ml)</td>
</tr>
<tr>
<td>Soluble fibrin monomer complex</td>
<td>(+)</td>
<td>(+)</td>
<td>negative</td>
</tr>
<tr>
<td>Plasminogen</td>
<td>21.0</td>
<td>35.0</td>
<td>63.0 - 105.0 (%)</td>
</tr>
<tr>
<td>α2-P I antigen</td>
<td>3.9</td>
<td>3.6</td>
<td>5.2 - 8.7 (mg/dl)</td>
</tr>
<tr>
<td>α2-P I–plasmin complex</td>
<td>4.6</td>
<td>1.4</td>
<td>&lt; 0.8 (µg/ml)</td>
</tr>
<tr>
<td>Antithrombin III activity</td>
<td>46.0</td>
<td>38.0</td>
<td>82.0 - 132.0 (%)</td>
</tr>
<tr>
<td>Antithrombin III antigen</td>
<td>10.0</td>
<td>7.0</td>
<td>15.0 - 30.0 (mg/dl)</td>
</tr>
<tr>
<td>Thrombin–antithrombin III complex</td>
<td>60.0</td>
<td>17.4</td>
<td>&lt; 3.0 (ng/ml)</td>
</tr>
<tr>
<td>Protein C activity</td>
<td>29.0</td>
<td>27.0</td>
<td>55.0 - 140.0 (%)</td>
</tr>
<tr>
<td>Plasminogen activator</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>inhibitor–1 antigen</td>
<td>618.0</td>
<td>260.0</td>
<td>&lt; 50.0 (ng/ml)</td>
</tr>
<tr>
<td>von Willebrand factor:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>antigen</td>
<td>559.0</td>
<td>260.0</td>
<td>50.0 - 155.0 (%)</td>
</tr>
<tr>
<td>Factor XIII</td>
<td>77.0</td>
<td>30.0</td>
<td>72.0 - 144.0 (%)</td>
</tr>
</tbody>
</table>

*N.E. = not examined.

* occur in patients with acute GVHD after allogeneic BMT; and (4) perivascular nuclear dust is frequently observed in skin biopsies from HLA mismatched recipients with acute GVHD, suggesting the immune-mediated endothelial cell damage in these patients.

In the present cases, it is of particular interest to note the marked elevation of plasma TNF-α and γ-IFN levels. The TA-GVHD is considered to be provoked by T-cells in the graft. In a murine model, monokine production stimulated by donor-derived T-cells have been implicated to play an important role in the pathogenesis of acute GVHD. The TNF-α, a monokine released by activated macrophages, can induce the expression of tissue factor on vascular endothelial cells and may, therefore, trigger the intravascular coagulation. The increase of plasma TNF-α in
DIC has been reported to be a pathogenic factor rather than a consequence of DIC and/or MOF.33

In an in vivo model of the Shwartzman reaction induced by bacterial lipopolysaccharide (LPS), of which the central pathological event is the development of DIC, γ-IFN released by activated T-cells has been proved to be one of the principal mediators.34 The γ-IFN has been known to enhance not only the production of monokines, such as TNF and IL-1, but also various responses of cells to them.35,36,37 Thus, γ-IFN may show a synergistic effect with TNF-α by enhancing the procoagulant activity on monocytes and vascular endothelial cells in the pathogenesis of DIC in TA-GVHD.
In addition, TNF-α and γ-IFN are known to stimulate the expression of class I and II antigens on vascular endothelial cells. Through GVH reaction, the recipients’ endothelial cells may have provided targets for activated donor T-cells, causing endothelial damage. These actions of TNF-α and γ-IFN may be important for the development of DIC in TA-GVHD. Holler et al have been reported the endothelial damage and intravascular clotting in acute GVHD after allogeneic BMT, speculating the similar mechanisms.

In spite of the extremely poor prognosis, no effective treatments for TA-GVHD have been established at present. Patient 2 was administered CsA in an attempt to suppress the functions of donor-derived T-cells but died of MOF shortly after the start of the CsA treatment. In vitro studies have demonstrated that CsA increases the procoagulant activity of monocytes and also enhances the cytokine-induced procoagulant activity of monocytes and vascular endothelial cells. Thus, there is a possibility that these actions of CsA deteriorated the DIC in Patient 2.

As TA-GVHD is a rare disorder, some in vivo model should be required to clarify a linkage between allogeneic GVH reactions and coagulopathies. In summary, one must reckon with DIC in the pathophysiology of rapid MOF progression in TA-GVHD; recognition of the conditions described would be helpful for the treatment of MOF in this disease.

References


