Post-Transfusion GVHD


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Abstract: The Japanese Red Cross Society has appointed representatives to Blood Centers nationwide to collect information on blood transfusion-associated adverse reactions, and data on 310 suspected cases of post-transfusion GVHD were collected during the 8-year period from 1993 to 2000. A diagnosis of post-transfusion GVHD was made in 61 cases based on the demonstrated presence of donor lymphocytes in the patients’ peripheral blood using a microsatellite analysis technique developed by the Japanese Red Cross Society. Case distribution was analyzed according to patient background characteristics. In a majority of the cases examined, transfusion was made from a homozygous donor of a HLA haplotype to a heterozygous patient of the specific HLA haplotype. The analysis of data also revealed that patients aged ≥70 and those undergoing transfusion for the first time were more liable to develop post-transfusion GVHD. Cells cloned from patient peripheral blood lymphocytes were assessed for their immunological characteristics to delineate subsets involved in GVHD. Analysis of these clones has led to the discovery of a drug (Futhan®; Torii Pharmaceutical) which suppresses their cytotoxic activity of these clones. The drug has proven to be effective both in vitro and in vivo.

Key words: Postoperative erythrodermia; Microsatellite; Post-transfusion GVHD; Irradiation (of blood for transfusion)

Introduction

In 1955, a paper entitled “On postoperative erythrodermia” was published in Geka (Surgery) by Toshimaru Shimoda1) of the Department of Surgery, Chiba University School of Medicine. The term “postoperative erythrodermia” was applied to a clinical condition characterized by serious symptoms including fever, erythema, and hepatic dysfunction that developed postoperatively in the twelve cases reviewed therein. Although the etiology remained unclear, Shimoda believed it to be multi-organ failure due to bacterial toxins. Six of the 12 patients died and the other 6 recovered. Leukocytopenia was noted in 5 of the 6 fatally affected patients. In retrospect, the clinical manifestations observed in the 5 docu-
mented cases were consistent with those of post-transfusion graft versus host disease (GVHD). Postoperative erythrodermia subsequently became the focus of growing concern as a serious adverse reaction of surgery, especially in the field of heart surgery.

Under such circumstances, Aoki et al. from the Department of Internal Medicine, Tsukuba University Hospital, reported a valuable case in the Journal of the Japanese Society of Internal Medicine (1984). The patient underwent a massive blood transfusion due to a gastrointestinal hemorrhage following surgery for an abdominal aortic aneurysm. Six days after the final transfusion, the patient developed fever and erythematous macules accompanied by liver dysfunction and diarrhea, and subsequently pancytopenia. Pathologic examination of bone marrow aspirates disclosed lymphocytes containing large granules adhering around the injured hematopoietic stem cells. The report has demonstrated that GVHD may be etiologically implicated in some cases previously diagnosed as postoperative erythrodermia.

The report has generated momentum for a reassessment of postoperative erythrodermia as GVHD, and active research has commenced in this field. The widely accepted theory that GVHD occurs only in immunodeficient patients has thus become subject to reexamination.

Reexamining Postoperative Erythrodermia as a GVHD

In their study conducted to examine the human leukocyte antigen (HLA antigen) complex in peripheral blood lymphocytes collected from patients, Sakakibara and Juji (1986) noted the existence of 2 patients bearing HLA antigens apparently distinct from those assumed to have been inherited, indicating that the patients’ own lymphocytes had been replaced with those from the blood donor.

Matsushita et al. of the Department of Pathology, Toranomon Hospital, found that lymphocytes isolated from skin lesions in 2 female patients diagnosed as having postoperative erythrodermia contained Y chromatin of male origin. This indicated that lymphocytes from a male blood donor had proliferated and mounted attacks upon various organs in these female patients.

An in-depth study of a case conducted using HLA antigens was reported by Ito et al. from the Department of Blood Transfusion, Kyoto University Hospital, in collaboration with Kyoto Blood Center of the Japanese Red Cross Society (JRC) (1988). They performed HLA typing of blood cell samples collected from the patient prior to and after the onset of postoperative erythrodermia. Two different HLA haplotypes assumed to be present from a genealogical examination were identified in the patient prior to onset. However, after the development of the disease, these two different HLA haplotypes were found to have been converted to homozygous with one of the haplotypes. Among this patient’s blood donors, one donor had identical homozygote of the haplotype to the post-onset haplotype found in the patient (i.e., homozygous HLA haplotype). The blood donor (AA)-patient (AX) combination in the case was thus unidirectionally compatible in terms of the HLA haplotype.

Thus, several studies in Japan have led to the formation of a consensus that some postoperative erythrodermia, if not all, are considered to be a post-transfusion GVHD.

Pathogenetic Mechanism of Post-Transfusion GVHD

Researchers in basic immunology discovered a new immunological phenomenon around the time when postoperative erythrodermia was reported by Shimoda in 1955. In an animal with radiation disorder, injection of splenocytes from another animal of the same species may be effective in treating the disorder, however, another disorder may develop after a period of time. This is a so-called secondary disease and is attributed to immune reactions against the
host caused by immunocompetent cells among the injected spleen cells, thereby mounting an attack on the host. Occurrence of acute GVHD after bone marrow allotransplantation was documented by Mathé et al. (1959) and after blood transfusion in immunologically deficient children by Hathaway et al. 5) (1965).

The following two conditions are noted in the development of GVHD: (1) Immunocompetent cells (chiefly, lymphocytes) injected extraneously are not precluded by the host, and (2) host tissue is recognized as a foreign body (i.e., possessing different histocompatibility antigens) by the injected immunocompetent cells.

Blood transfusion into immunodeficient (deficient in cell-mediated immunity) patients would fulfill the above conditions. In such case, immunocompetent cells contained in transfused donor blood are incapable of excluding any HLA types; hence the patients are vulnerable to immunologic attacks. When blood from an HLA haplotype homozygous donor (A24, B52 and DR15 is the commonest haplotype among Japanese) is transfused into a heterozygous patient of the haplotype and other haplotypes than the haplotype, the patient is unable to recognize immunocompetent cells contained in the transfused donor blood as foreign bodies and is competent for those cells even if the patient is not immunologically deficient. These cells recognize HLA antigens of the non-public haplotype distributed in patient tissues as foreign bodies and may eventually launch an attack.

Nationwide Survey on Incidence of Post-Transfusion GVHD

In view of the high frequency of suspected post-transfusion GVHD events reported among heart surgery cases, in 1986 the Japanese Society of Blood Transfusion in cooperation with the Japanese Society of Thoracic Surgery collected clinical data on suspected cases of post-transfusion GVHD encountered among 63,257 cases treated by open heart surgery at 137 hospitals nationwide during the 6-year period from 1981 to 1986. An analysis of the data revealed that post-transfusion GVHD was diagnosed in 96 patients; i.e. an incidence rate of 1 per 658.9 patients with open heart surgery.6) A nationwide survey conducted by the JRC Research Group in 1991 revealed the frequent occurrence of post-transfusion GVHD not only among cases of cardiovascular surgery but among cases of surgery for malignancies as well.

Method for Definite Diagnosis of Post-Transfusion GVHD

It is essential to demonstrate the presence of donor-derived lymphocytes in the patient peripheral blood at a certain level, using a small quantity of blood sampled from the patient. In general, it is considered risky to unduly raise sensitivity levels in terminal-stage patients with pancytopenia because they are receiving blood transfusions on consecutive days. As far as the cases we have examined are concerned, replacement of peripheral blood lymphocytes with lymphocytes of donor origin was evident in the majority of patients with post-transfusion GVHD. We have established and reported a laboratory testing method which utilizes the fact that the number of repetitions of the gene base sequence for microsatellite markers varies among individuals (1994).7)

This test requires the use of a nail specimen and a small volume of blood sampled from the patients. Lymphocytes from a donor do not infiltrate the nails even if post-transfusion GVHD has developed. The electrophoretic pattern of DNA extracted from the nail, being taken as patient-derived DNA, is compared with that of DNA from circulating blood lymphocytes. Care is taken to avoid incidental concordance by selecting five highly polymorphic microsatellite sites for the assay.
Drug Information Activities of the Japanese Red Cross Society

Beginning in 1993, the Japanese Red Cross Society has been appointing drug information officers to Blood Centers nationwide to collect information on adverse reactions to blood transfusion, and has analyzed the information collected. Accordingly, any data considered to be of significance has been reported to the Ministry of Health, Labor and Welfare.

Cases of suspected post-transfusion GVHD found have been subjected to the above microsatellite test since 1993 (Fig. 1). A definite diagnosis of post-transfusion GVHD was made in 9 of the 32 cases with relevant specimens reported in 1993. During the period from 1993 to 2000, 310 cases with relevant specimens were reported as suspected cases of post-transfusion GVHD to the JRC Central Blood Center from medical institutions across the country, with a diagnosis of post-transfusion GVHD being established in 61 cases based on microsatellite test data.

Analysis of Background Factors in 61 Definitely Diagnosed Cases

Gender difference: Thirty-eight male and 23 female cases were definitely diagnosed with GVHD; there was no statistically significant difference between the sexes.

Aging: Of the 61 patients, 31 were aged ≥70 years while patients at 59 years or younger numbered fewer than 6. When age distribution is compared with the number of blood transfusion units used in the Tokyo Metropolitan Area, nationwide data revealed a significant difference for the ≥70 years age group at $p<0.01$, indicating that aging constitutes a risk factor for post-transfusion GVHD.

Blood transfusion history: Fifty-one of the 53 patients, excluding 8 cases with no documented blood transfusion history, experienced GVHD following the first transfusion. It has been suggested that resistance to post-transfusion GVHD may be induced in patients undergoing repeated blood transfusions. Laboratory animal study data supporting this hypothesis have also been reported. The primary disorders for which surgery was performed included malignant tumors in 25 cases, cardiovascular disorders in 13 cases, and traumas in 9 cases. In 2 patients who received blood transfusion for massive hemorrhage from a gastric ulcer, GVHD developed after the transfusion alone without surgical intervention.

Patient-Donor Combination of HLA Antigens

In a majority of the cases examined, the donor was homozygous with a haplotype: HLA-A24, B52 and DR15 while the patient was heterozygous with the said haplotype plus a different haplotype. Cases also included a combination of a haplotype homozygous donor (A33, B44 and DR13), ranking in second place in terms of frequency, and a heterozygous patient. There were a donor-patient combination where the HLA-A and HLA-DP antigen series were homo-/heterologous and 2 combinations where only the HLA-DR antigen series was homo-/heterologous. In cases of severe combined immunodeficiency, the condition has no specific relationship with patient
and donor HLA antigens, hence transfusion of blood from any other individual may lead to the development of GVHD.

Establishment of Cell Clones from Peripheral Blood Lymphocytes

As it is inferred that donor-derived immunocompetent cells in the blood of a patient mount an attack on host tissues, we attempted to clone such cells to analyze their characteristics. Cell clones were prepared from lymphocytes of a total of 5 patients. The target antigens for the CD8<sup>+</sup> cytotoxic T cell clones were found to be class I antigens such as HLA-B46 and B52. The target antigens for the CD4<sup>+</sup> cytotoxic T cell clones were noted to be class II antigens such as HLA-DR4, DR13, DR15, and DP4. Another CD4<sup>+</sup> clone obtained had no direct cytotoxic activity, responded with proliferation to antigen stimulation and produced tumor necrosis factor (TNF). A B cell clone elaborating an antibody which reacts with HLA-DR4 antigen was also obtained.8)

Treatment of Post-Transfusion GVHD

An attempt was made to seek drugs for treatment of post-transplantation GVHD using the cytotoxic cell clones proven to react with target antigens <em>in vitro</em>. The exploration was conducted using commercially available drugs insomuch as it was impracticable to try new drugs in patients with post-transplantation GVHD where death usually follows within 1–2 weeks of the onset of clinical symptoms.

Involvement of perforin and granzymes constitutes, at least in part, a mechanism whereby cytotoxic T cells mount an attack on target cells. Perforin makes a hole in the cell membrane of target cells, and granzyme injected by the T cell injures the target cells via its enzymatic activity. The granzyme exhibits serine protease activity during the cytotoxic process. Futhan<sup>®</sup> (nafamostat mesilate), which inhibits the serine protease activity and has been used to treat disseminated intravascular coagulation syndrome (DIC) was discovered to markedly suppress the activity of a cytotoxic T cell clone.9) FOY<sup>®</sup> (gabexate mesilate), another drug used for the treatment of DIC, was demonstrated to be entirely ineffective in this respect (Fig. 2).

Futhan<sup>®</sup> was administered to 4 patients with post-transplantation GVHD, all of whom responded with symptomatic amelioration. Analyses by the microsatellite method revealed that peripheral blood lymphocytes returned from donor-predominant to the patient’s intrinsic population.10) Nevertheless, plasma potassium elevation may occur as an adverse reaction inherent to the use of Futhan<sup>®</sup>. In the first 3 cases, the Futhan<sup>®</sup> medication was discontinued as plasma potassium increased to &gt;6 mEq/l, and subsequently GVHD recrudesced, fatally affecting the patients. In the fourth case, the treatment with Futhan<sup>®</sup> was continued while appropriate measures were adopted to control the plasma potassium level, to effectively suppress the recurrence of GVHD over an extended period. This patient died due to a cause other than GVHD. The results in these cases, and <em>in vivo</em> experiments, have demonstrated that Futhan<sup>®</sup> effectively suppresses the activity of cytotoxic T cells.

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Fig. 2  Suppressive effects of drugs on cytotoxic activity
more effective treatment will be established if any measures to eliminate activated cytotoxic T cells are introduced in line with the above suppressing effect of Futhan®.

**Prevention of Post-Transfusion GVHD**

Blood products supplied by JRC Blood Centers are pretreated with radiation when directed to do so by the medical institution requesting such products. Non-irradiated blood products are supplied to medical institutions furnished with radiation units. However, the use of non-irradiated blood for transfusion has fallen dramatically in line with increasing concern about GVHD associated with blood transfusions. Consequently, to our delight, there were no definitely diagnosed cases of post-transfusion GVHD among the suspected cases collected by the Japanese Red Cross Society in 2000 (Fig. 1), and also none among the suspected cases collected up to October 2001.

**Conclusion**

Japanese researchers have demonstrated that post-transfusion GVHD, which had been diagnosed as postoperative erythrodermia and regarded as a symptom of unknown etiology since 1955, occurs even in the absence of immunodeficiency. Furthermore, the studies have indicated that the relatively high incidence of post-transfusion GVHD in Japan was at least partially attributable to the high uniformity of HLA antigen complex and the high proportion of homozygous HLA haplotypes among indigenous blood donors.

The advent of the microsatellite method contributing to the feasibility of definite diagnosis is also a noticeable progress. The etiology accounting for the failure to detect donor lymphocytes in the peripheral blood of patients suspected to have post-transfusion GVHD is yet to be clarified.

Long-term observation is to be pursued to ascertain whether irradiated blood transfusions are truly safe or not.

**REFERENCES**


