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Review

TRANSFUSION-ASSOCIATED GRAFT-VERSUS-HOST DISEASE

Transfusion-associated graft-versus-host disease (TA-GVHD) is an infrequent, although nearly uniformly fatal, complication of blood transfusion. Graft-versus-host disease (GVHD) was first described in animals as a result of the injection of immunologically competent cells into a host that, for unknown reasons, was unable to reject them (Billingham, 1966). This disease was reported in humans in 1959 after allogeneic bone marrow transplantation (BMT) (Mathé *et al*, 1959). Since that time, GVHD has become a well-known complication of BMT with its clinical presentation of fever, skin rash, liver dysfunction and/or diarrhoea. It was not until the 1970s and 1980s that TA-GVHD was defined as a disease entity, first in immunocompromised and later in immunologically competent individuals. Although there are many similarities between GVHD secondary to BMT and transfusion, TA-GVHD is associated with marrow aplasia and therefore has a more rapid and fulminant course, nearly always resulting in the death of the patient.

The true incidence of this disease is unknown, as the diagnosis of TA-GVHD is easily missed because other conditions, such as a viral infection or drug reaction, may have similar clinical features. The physician must have a high index of suspicion and associate the clinical picture with a recent transfusion. This review will cover the mechanism, risk factors, clinical and pathological picture, diagnosis, treatment and, most importantly, prevention of TA-GVHD.

METHODS

A search of Medline articles published since 1960 was performed using the key words: graft-versus-host disease and blood transfusion. However, articles and abstracts published in English were reviewed primarily and these are believed to contribute adequately to our knowledge of this disease. A significant number of publications were in Japanese because of the high prevalence of TA-GVHD in that country. It is of interest to note that the majority of papers were published between 1985 and 1996. This may represent, at least in part, successful prevention of this disease.

HISTORY

In 1916, Murphy reported a syndrome in chicks after the injection of cells from adult chicken spleens and bone marrow into chick embryos; he noted the development of an enlarged spleen and disseminated nodules in these chicks. In the 1950s, Simonsen undertook similar experiments in both chickens and mice and interpreted these studies as being consistent with GVHD. At the same time, Billingham and Brent described a disease termed 'runt disease' in mice after the injection of allogeneic spleen or bone marrow cells into newborn mice. The mice developed skin lesions, diarrhoea, wasting and ultimately died. This syndrome was thought to be the result of a graft-versus-host reaction (Billingham, 1966). In 1959, Mathé reported what he described as 'secondary syndrome', now known to be GVHD, in patients who had undergone bone marrow transplantation. These patients developed symptoms similar to those seen in mice, namely, skin rash, diarrhoea, liver dysfunction and subsequent death (Mathé *et al*, 1960).

Shimoda (1955) described a condition he called 'post-operative erythroderma' (POE) which is now thought to be the first report of TA-GVHD. He reported 12 patients who developed a skin rash and high fever between 6 and 13 d after surgery. Six of these patients died while the other six survived after treatment including antibiotics and steroids. Although he did not discuss the transfusion history of each patient, he did state that transfusions were given pre- and postoperatively with fresh blood. It was common practice at that time for surgeons to transfuse essentially all their patients with fresh blood that had not been banked (T. Juji, personal communication). This observation and the clinical picture led Aoki *et al* (1984) to conclude that POE and TA-GVHD were the same disease.

In 1965, a similar syndrome was described in two children with congenital immunodeficiency who developed severe progressive vaccinia necrosum after a routine smallpox vaccination. One child was treated with fresh leucocyte-rich plasma and the other child with exchange transfusion using fresh whole blood from recently vaccinated donors. Both children rapidly developed a skin rash, hepatomegaly and pancytopenia, and died. The clinical picture was thought to be consistent with that of runt disease seen in animals, as well as that of secondary syndrome reported after allogeneic bone marrow transplantation (Hathaway *et al*, 1965).

MECHANISM OF GVHD

Billingham (1966) defined the three main requirements for the development of GVHD. These are:

''1. The graft *must* contain immunologically competent cells.

Correspondence: Marlis L. Schroeder, MD, FRCPC, Professor, Department of Pediatrics and Child Heath, University of Manitoba, 2011–675 McDermot Avenue, Winnipeg MB, Canada R3E 0V9. E-mail: Marlis.Schroeder@cancercare.mb.ca

2. The host must possess important transplantation alloantigens that are lacking in the donor graft, so that the host appears foreign to the graft and is therefore capable of stimulating it antigenically.

3. The host itself must be incapable of mounting an effective immunological reaction against the graft, at least for sufficient time for the latter to manifest its immunological capabilities; that is, *the graft must have the security of tenure*.''

In routine transfusion practice, the first two requirements are usually present; the major variable is the immune status of the recipient. The human leucocyte antigen (HLA) match between donor and recipient is nearly always incompatible because no attempt is made to match for the antigens of the major histocompatibility complex (MHC). All cellular blood products contain mature T cells, the immunocompetent cells that mount the GVHD response. The donor cells are rejected if the recipient is not immune suppressed. However, in the presence of an underlying immune deficiency, either congenital or secondary to chemotherapy and/or radiotherapy, the recipient cannot reject the foreign T cells, which proliferate resulting in a GVH reaction and the clinical picture of TA-GVHD.

TA-GVHD may also develop in immune-competent individuals if the last requirement is not met, that is, if the recipient is 'incapable of mounting an effective immunological reaction against the graft.' This occurs when the donor and recipient share HLAs or a haplotype. The recipient does not recognize the transfused donor cells as foreign and cannot reject them. The transfused, viable T cells are capable of mounting a GVH response; they react against the second haplotype or HLAs that are not shared, with the resulting clinical picture of TA-GVHD.

The development of acute GVHD after BMT can be divided into three separate phases: phase one is the conditioning regimen which results in tissue damage and activation of host tissues with the resultant production of inflammatory cytokines. Phase two, the afferent phase, results in T-cell activation, and phase three, the efferent phase, consists of the release of inflammatory cytokines. The second or afferent phase consists of three steps: antigen presentation resulting in T-cell activation, followed by proliferation and then differentiation of activated T cells into cells that are cytolytic or that secrete cytokines. The efferent phase appears to be mediated primarily by cytokines which attack host tissues either directly or through the recruitment of haematopoietic cells such as natural killer (NK) cells, macrophages or T cells, resulting in cell death and host tissue destruction (Ferrara & Deeg, 1991; Ferrara & Antin, 1999).

The mechanism of TA-GVHD appears to be the same. Nishimura *et al* (1997) characterized T-cell clones from a patient who developed TA-GVHD postoperatively after transfusion of stored red cells and fresh platelets from his son and daughter. The diagnosis of TA-GVHD was confirmed by the analysis of microsatellite DNA polymorphisms and by HLA typing. The son was found to be the responsible donor. The HLA genotype of the patient was: A*2402, B*4002, B*52011, DRB1*1502 and DRB1*0901 (serologically A24, B61, B52, DR15 and DR9). The son typed serologically as A24, B38, B61 and DR9. HLA B52 and DR15 were the two paternal antigens not shared by the son. The authors established nine T-cell clones from the patient's peripheral blood leucocytes which, through microsatellite analysis, were shown to originate from the son. Three types of T-cell clones were identified: type I was $CD8^+$ and lysed the patient's HLA B52-positive cells; type II clones expressed CD4, proliferated in response to and lysed cells expressing DR 15; type III, a non-cytotoxic $CD4^+$ clone, produced and secreted tumour necrosis factor β after antigen stimulation. Two of the clones, a $CD8^+$ and a $CD4^+$, were cytolytic, while the third, a $CD4^+$ clone, secreted cytotoxic lymphokines (Nishimura *et al*, 1997). These studies helped to confirm that the phases in the development of GVHD after transfusion are similar to those seen in GVHD after BMT.

GROUPS AT RISK

There have been many case reports and reviews describing this disease in both immunocompromised and immunocompetent patients (Anderson & Weinstein, 1990; Greenbaum, 1991; Ohto & Anderson, 1996a,b; Strauss, 2000).

TA-GVHD in immunocompromised patients

1. Congenital immunodeficiency syndromes. These children remain at high risk of developing this disease because they may be transfused before the diagnosis of immunodeficiency is made. TA-GVHD has been reported not only in children with severe congenital immunodeficiency syndromes (SCIDS), as reviewed recently by Strauss (2000), but also in some of the variable immunodeficiency syndromes such as Wiskott–Aldrich syndrome (Douglas & Fudenberg, 1969) and purine nucleoside phosphorylase deficiency (Strobel *et al*, 1989).

2. Fetuses and newborns. Neonates are known to have an immature immune system, however, the degree to which this places them at risk of TA-GVHD remains controversial. Pre-term babies have a more immature immune system than those born at term. Their underlying immaturity is further compromised by other factors such as transfusions, surgery and nutritional status that may affect their immune status. This high-risk group of patients is most likely to receive transfusions from family members, that is, directed donations. TA-GVHD in this patient population is nearly always associated with directed donations (Ohto & Anderson, 1996a; Strauss, 2000).

- Intrauterine and exchange transfusions: an intrauterine or exchange transfusion represents a large volume transfusion of fresh blood to a relatively immature host. Although infrequent, TA-GVHD has been reported after intrauterine transfusions and exchange transfusion for newborns with erythroblastosis fetalis (Naiman *et al*, 1969; Parkman *et al*, 1974; Hentschel *et al*, 1995). Irradiation is recommended for all intrauterine transfusions as fresh blood is used and these transfusions are always performed on an elective basis. Irradiation of the product will not result in a delay in treatment. With exchange transfusions, on the other hand, the primary focus should be on delivery of optimum care to the newborn. If irradiation of the product would result in undue delay of

therapy, exchange transfusion should proceed regardless if clinically indicated. Other risk factors, such as the possibility of an immune deficiency in the newborn, must be considered before proceeding.

- Pre-term infants: although preterm infants are frequently transfused, only seven cases of TA-GVHD (three from Japan) have been reported in preterm infants who received transfusions, including exchange transfusions, from random blood donors (Seemayer & Bolande, 1980; Wise & Lawrence, 1990; Funkhouser *et al*, 1991; Ohto & Anderson, 1996a; Strauss, 2000). All other cases have occurred after transfusion from family members (Berger & Dixon, 1989; Ohto & Anderson, 1996a). The risk of TA-GVHD is very small in the preterm infants who are transfused from random blood donors.
- Term infants: the risk of TA-GVHD does not appear to be increased in healthy full-term newborns transfused from random blood donors, in spite of the perception that they may be more likely to develop this complication.

3. Patients with haematological malignancies. The majority of cases of TA-GVHD have been reported in patients with haematological malignancies (Kessinger *et al*, 1987). Patients with Hodgkin's disease, with its associated immune deficiency state, are at highest risk of developing this disease (Dinsmore *et al*, 1980; von Fliedner *et al*, 1982; Burns *et al*, 1984; Decoste *et al*, 1990). Patients with other diseases, such as the acute leukaemias, that are treated with intensive chemotherapy have been reported to develop TA-GVHD (Lowenthal *et al*, 1981; Nikoskelainen *et al*, 1983). In part, the risk may be higher in these patients because of the requirement of intensive platelet support with the use of HLA-matched single donor platelet transfusions. Patients with non-Hodgkin's lymphoma appear to be at lesser risk, but cases of TA-GVHD have been reported (Saab *et al*, 1983; Mutasim *et al*, 1984; Spitzer *et al*, 1990; Gelly *et al*, 2000). TA-GVHD has been reported more recently in patients with chronic lymphocytic leukaemia who have been treated with one of the highly immunosuppressive purine analogues, e.g. fludarabine (Maung *et al*, 1994; Williamson *et al*, 1996).

4. Patients with solid tumours. TA-GVHD has been reported in patients with solid tumours, including neuroblastomas (Woods & Lubin, 1981; Kennedy & Ricketts, 1986), rhabdomyosarcoma (Labotka & Radvany, 1985), bladder cancer (Saito *et al*, 1993) and small cell lung cancer (Spector, 1995), to name a few. Therapy for patients with solid tumours has changed and has become more dose intense, more immunosuppressive and myeloablative. These patients are requiring more transfusions and are therefore at higher risk of developing TA-GVHD.

5. Patients undergoing bone marrow transplants:

Allogeneic: blood and blood products have been irradiated routinely for patients undergoing allogeneic BMT, since the first report by (Thomas *et al*, 1961).

Autologous: for patients undergoing autologous BMT, irradiation of blood and blood products has not been routine practice in many centres. Therefore, TA-GVHD has been reported in such patients undergoing autologous BMT for conditions such as leukaemia, germ cell tumour and lung cancer (Postmus *et al*, 1988).

6. Patients after solid organ transplantation. TA-GVHD is a rare complication in patients who have undergone a solid organ transplant even though these individuals are both highly immunosuppressed and multiply transfused. In this patient population, GVHD is usually caused by the proliferation of lymphocytes from the transplanted organ and not as a result of transfusion (Triulzi & Nalesnik, 2001). However, TA-GVHD has been reported after heart (Sola *et al*, 1995) and liver (Wisecarver *et al*, 1994) transplants. The source of the lymphocytes, organ donor or blood donor, must be determined to differentiate between the two types of GVHD.

7. Patients with acquired immune deficiency syndrome (AIDS). AIDS is not considered a risk factor for the development of TA-GVHD (Mayer, 1990; Anderson *et al*, 1991; Popovsky *et al*, 1995). There is only one reported case of a child with AIDS developing TA-GVHD, from which she recovered (Klein *et al*, 1996). The reasons for this are probably multifactorial.

- The diagnosis of TA-GVHD is easily missed, especially in patients who may have similar symptoms, such as rash, diarrhoea, liver dysfunction or pancytopenia, that are related to their underlying disease and/or treatment.
- Many of these patients are already receiving irradiated blood. A survey of irradiation practice in the United States (US) in 1991, revealed that 15·9% of the institutions routinely provided irradiated blood products to patients with this diagnosis (Anderson *et al*, 1991). This practice is probably in even more common use at the present time.
- TA-GVHD may not develop because the transfused donor lymphocytes become infected with the human immunodeficiency virus (HIV); they are then incapable of mounting a GVH response.

Table I summarizes groups of patients at risk of developing this syndrome. They have been divided, somewhat arbitrarily, into 'significantly increased', 'minimally increased' and 'no reported increased' risk of GVHD. The risk of developing TA-GVHD is not quantifiable in any of the categories because the information on the total number of patients transfused, the number of transfusions and the type of blood product is not available. The risk, whether high or low, is deduced from the number of case reports in each of the disease groups. This risk is dependent on the type and dose intensity of immunosuppressive or chemotherapeutic agents administered. Each transfusion service must develop guidelines for prevention in accordance with local risk factors.

TA-GVHD in immunocompetent recipients

The first case report of TA-GVHD in an immunocompetent individual was from Japan (Aoki *et al*, 1984); a patient developed a fever, skin rash, diarrhoea and pancytopenia after cardiac surgery and subsequently died. This disease was thought to be the same as that reported several decades earlier by Shimoda (1955), which was called 'postoperative erythroderma'. It resembled GVHD both clinically and pathologically.

POE was later confirmed as being TA-GVHD by the demonstration of a change in the patient's HLA phenotype

Table I. Risk factors for the development of TA-GVHD.

Significantly increased risk
Congenital immunodeficiency syndromes
Bone marrow transplantation
Allogeneic and autologous
Transfusions from blood relatives
Intrauterine transfusions
HLA-matched platelet transfusions
Hodgkin's disease
Patients treated with purine analogue drugs
Minimally increased risk
Acute leukaemia
Non-Hodgkin's lymphoma
Solid tumours treated with intensive chemotherapy or radiotherapy
Exchange transfusions
Pre-term infants
Solid organ transplant recipients
Perceived but no reported increased risk
Healthy newborns
Patients with AIDS

to that of the blood donor. The change was deduced in the first two cases by determining that the patients' HLA phenotypes were not consistent with family typing (Sakakibara *et al*, 1989). Ito *et al* (1991) subsequently demonstrated that a patient's HLA class I phenotype changed from his own on d 16 post transfusion to that of one of his blood donors on d 17. HLA class II antigens were present on both the patient's T and B cells, suggesting T-cell activation. These findings are consistent with GVHD and provide direct evidence of donor cell proliferation in patients with this clinical picture (Haga *et al*, 1989; Otsuka *et al*, 1989; Ito *et al*, 1991).

The report by Thaler *et al* (1989) from Israel helped to elucidate the mechanism of TA-GVHD in immunocompetent recipients. The authors described two cases of fatal TA-GVHD in immunocompetent individuals after cardiac surgery. Both patients received fresh, non-irradiated whole blood from their children. In each case, one of the donors was HLA-homozygous and shared one haplotype with the recipient who could not reject the HLA-homozygous cells as these were not recognized as foreign. The transfused, viable, donor lymphocytes, on the other hand, recognized the host as foreign, proliferated and induced a graft-versus-host response.

There have been numerous reports in the literature of TA-GVHD in immunocompetent patients, the majority again from Japan or in patients transfused from family members (Petz *et al*, 1993; Ohto & Anderson, 1996b).

BLOOD PRODUCTS ASSOCIATED WITH TA-GVHD

All cellular blood products, including red cells, platelet and granulocyte concentrates, and even fresh plasma, contain viable, immunocompetent T lymphocytes. All of these products have been implicated in TA-GVHD.

RISK FACTORS

The incidence of TA-GVHD appears to be highest in Japan. Ohto & Anderson (1996b) reviewed the cases of TA-GVHD in immunocompetent patients from Japan in an attempt to reveal additional factors that may predispose to this disease. They discussed 122 cases in detail and divided them into three different groups according to their underlying disease: (1) patients undergoing cardiovascular surgery (56 patients), (2) patients with solid tumours treated only by surgery (39 patients), and (3) a miscellaneous group of patients requiring transfusion with diagnoses such as peptic ulcers, fractures, cholecystitis and trauma (25 patients). In all three groups the clinical syndrome, median time of onset and eventual outcome were similar, with only two patients surviving, all in group 3. Of the 30 patients in whom TA-GVHD was confirmed by HLA typing, donor and patient shared at least one haplotype; 28 (93%) of these donors were HLA-homozygous for the shared haplotype, with the A24 B52 haplotype implicated in more than half the cases. The high frequency (9·2%) of this haplotype in the Japanese population may account for the high incidence of TA-GVHD. The risk of receiving a homozygous donor in the Japanese population is 1 in 874, a risk that increases 8–30-fold if an in-family blood donor is used. This risk compares with 1 in 7174 for unrelated individuals and 1 in 475 for first-degree relatives in the USA (Ohto *et al*, 1992). Further risk analysis of TA-GVHD due to homozygous HLA haplotypes has placed the range of the risk for non-directed transfusion at 1 in 17 700–39 000 in US whites, at 1/6 900–48 500 in Germans and 1/1 160–7900 in Japanese. With directed donations the risk increases at least 21-fold for US whites, 18-fold for Germans and 11-fold for Japanese (Wagner & Flegel, 1995). The genetic homogeneity of the Japanese population places them at significantly greater risk of developing TA-GVHD.

Different transfusion practices may also play a role in the reported increased prevalence of TA-GVHD in Japan compared with other countries such as the US. In Japan, fresh blood which may be 'warm', that is, never refrigerated or < 24 h old, as well as directed donations, were commonly used for transfusion in patients undergoing coronary artery bypass graft surgery. This contrasts with the North American practice in which stored blood is normally used and directed donations are reported at < 2% in this patient population (Goodnough *et al*, 1990). In their review of Japanese cases, Ohto & Anderson (1996b) reported that 62% of patients with TA-GVHD received fresh blood, which they defined as < 72 h old. Petz *et al* (1993) reported that, similarly, in about 90% of cases of TA-GVHD in the US, the transfused blood was < 4 d old. The use of 'fresh blood' is an additional factor that places patients at risk of developing TA-GVHD.

Such reports have led investigators to study whether changes that may occur in donor leucocytes during storage decrease the risk of developing TA-GVHD. Mincheff (1998) has shown that, after 2 weeks of storage, leucocytes progressively undergo apoptosis and fail to stimulate and respond in a mixed leucocyte culture (MLC). Similarly,

Chang *et al* (2000) have shown that, by d 3 of storage, the cells are less responsive in MLC, while by d 5 the response to phytohaemagglutinin (PHA) and in MLC is abrogated. These findings are consistent with the reports that most cases of TA-GVHD are seen in patients who have been transfused with fresh blood. There are, however, exceptions and, although rarely, TA-GVHD has been reported in patients who have been transfused with blood stored for longer than 7 d.

The risk of TA-GVHD from other cellular products such as platelets, transfused within 5 d, and granulocytes, transfused within 24 h of collection, remains. TA-GVHD has been reported after transfusion of HLA-matched non-irradiated platelets from unrelated HLA-homozygous donors (Benson *et al*, 1994). Granulocyte transfusions have been most frequently implicated in TA-GVHD; these components are transfused fresh, have a high lymphocyte count and usually are administered to neutropenic and immunosuppressed patients (Perkins, 1981). TA-GVHD has been reported after granulocyte transfusions, collected either from normal donors (Ford *et al*, 1976; Weiden *et al*, 1981; Weiden, 1984), from patients with chronic myelogenous leukaemia (Graw *et al*, 1970; Lowenthal *et al*, 1975) or from family donors (Tolbert *et al*, 1983).

CLINICAL PICTURE

The clinical presentation of TA-GVHD is similar to that seen in GVHD after BMT. There are, however, several distinct differences, namely the time of onset, the presence of marrow hypoplasia and the course of the disease. In both groups of patients, the classic symptoms include a fever, a rash, liver dysfunction and diarrhoea. In TA-GVHD, the onset is earlier. Fever (> 38°C) is usually the presenting symptom and may occur as early as 4 d post transfusion, with a median onset of 10 d (Ohto & Anderson, 1996b). Next is an erythematous, maculopapular skin rash that usually begins on the trunk and then extends to the extremities, including the palms of the hands and the sole of the feet. This rash may be mild or there may be a generalized erythroderma that may progress to bullous lesions. Clinically, this rash is indistinguishable from that seen after BMT. The degree of liver dysfunction is variable. The most common picture is consistent with an obstructive jaundice with elevated bilirubin and alkaline phosphatase associated with abnormal liver enzymes, although usually not to the extent seen in acute hepatitis. Similarly, the gastrointestinal complications are variable and range from anorexia and nausea to massive diarrhoea. The leucopenia and pancytopenia associated with TA-GVHD are later developments (median 16 d) and become progressively more severe. Overwhelming infections are the most common cause of death, which frequently occurs within 3 weeks of the onset of symptoms. The mortality rate is > 90%.

In neonates, the clinical picture is similar to that seen in adults; however, the onset is delayed. The most comprehensive report of TA-GVHD in newborns is from Japan with a review of over 30 cases and a detailed analysis of 27 neonates (20 premature and 7 full-term) (Ohto & Anderson, 1996a). Of these, 10 had received exchange transfusion (8 with blood from a family member), two received transfusions peri-operatively and the remainder were transfused for a variety of indications. Fever (> 38°C) was the presenting symptom, with a median time of onset of 28 d after transfusion compared with 10 d in the adult. A skin rash (median 30 d) with the characteristic pathological findings occurred next, followed by leucopenia (median 43 d). All 27 infants died (median 51 d), despite efforts at therapy similar to that used in the treatment of TA-GVHD in adults. Infection, bacterial, fungal or viral (cytomegalovirus), was the primary cause of death. The most common risk factor in 23 of the 27 infants was the transfusion of fresh whole blood administered within 72 h of donation; 22 patients received blood from relatives. Only five patients received blood solely from unrelated community donors.

The diagnosis is missed more easily in neonates than in adults. Skin rashes are very common for other reasons, especially in premature infants, occurring in 9–12% of them. Skin erythema is common because of the use of incubators to maintain the infant's body temperature and the use of phototherapy; the significance of any redness or skin rash may therefore be underestimated. Similarly, the long median time interval (4 weeks) between transfusion and clinical signs of TA-GVHD delays or even prevents consideration of the diagnosis, because the clinical manifestations of TA-GVHD are attributed to the underlying illness or to prematurity.

LABORATORY AND PATHOLOGICAL FINDINGS

The diagnosis of TA-GVHD is made through the association of clinical manifestations combined with relevant laboratory findings. The latter may reveal the presence of leucopenia and pancytopenia as well as abnormalities in liver function tests. Other relevant investigations may include a skin, liver and/or a bone marrow aspirate/biopsy. Characteristic changes in the skin may include epidermal basal cell vacuolization (grade I); a mononuclear cell infiltration in the epidermis and degeneration of the epidermal basal layer (grade II); bulla formation (grade III); and ulceration of the skin (grade IV). Grade I and II GVHD of the skin is most common. The liver is commonly involved with the small interlobular and marginal bile ducts, the preferential target of the immune reaction. The liver may show degeneration of the small bile ducts and periportal mononuclear infiltrates associated with hepatocellular and cholangiolar cholestasis (Sale *et al*, 1999). The bone marrow may be hypocellular or aplastic with a lymphocytic or histiocytic infiltration. There may also be evidence of haemophagocytosis.

HLA typing, either serologically or by DNA analysis, is essential in the investigation of TA-GVHD. The demonstration of donor cells or DNA in the patient's circulation or in cellular infiltrates in association with the clinical picture confirms the diagnosis of TA-GVHD. The identification of additional or different HLA antigens to those in the patient confirms the engraftment of the transfused cells. These results can be compared with the HLA type of the implicated

donors. Donor DNA can be obtained from blood or from cellular infiltrates. However, pure host DNA may not be easily obtained from blood because of aplasia and donor engraftment. Alternative tissues such as skin fibroblasts, hair or even fingernails have been proposed as a source of host DNA as these are not 'contaminated' by donor cells (Uchida *et al*, 1996). If the patient cannot be typed, the HLA type of that individual may be deduced by typing family members. However, with polymerase chain reaction (PCR)-based methods, HLA typing of peripheral blood is usually feasible.

Other methods to determine the presence of donor cells in the patient include the comparison of restriction fragment length polymorphisms (DePalma *et al*, 1994), variable number tandem repeat (VNTR) analysis and human microsatellite markers (Wang *et al*, 1994; Briz *et al*, 1995; Warren *et al*, 1999), and/or cytogenetic analysis of host and graft cells (Kunstmann *et al*, 1992; Hayakawa *et al*, 1993; Otsuka *et al*, 1994).

The presence of donor lymphocytes alone without the clinical picture is not indicative of TA-GVHD. Mixed chimaerism, that is the presence of both host and donor haematopoietic cells, has been reported in patients who have undergone allogeneic bone marrow transplants and who are otherwise well. Similarly, microchimaerism (< 2·5% donor cells) has been seen in recipients of solid organ transplants. A state of tolerance has developed in these individuals. There is no evidence of GVHD (Storb *et al*, 1999).

The normal clearance of donor lymphocytes has been investigated by Lee *et al* (1995), who studied the kinetics of donor leucocytes after transfusion of packed red cells in otherwise healthy individuals undergoing orthopaedic surgery. They demonstrated several phases: first, a clearance of the majority (99·9%) of donor leucocytes within the first 2 d, followed by an increase in circulating donor leucocytes on d 3–5 and, finally, a secondary clearance of donor cells on d 5–7. They postulated that this increase in circulating donor leucocytes was the result of an *in vivo* two-way mixed leucocyte reaction or a graft-versus-host and a graft-rejection phenomenon. If the host is immunoincompetent or cannot reject the transfused cells because of HLA haplo-identity, as discussed previously, the leucocytes continue to proliferate unchecked and cause TA-GVHD.

A diagnosis of TA-GVHD must include both the clinical features as well as evidence of blood donor lymphocyte engraftment.

TREATMENT

Attempts at treatment of TA-GVHD are largely ineffective. In contrast with GVHD post BMT, these patients do not respond to corticosteroids, antithymocyte globulin, cyclosporine and/or growth factors. However, there are reports in the literature of spontaneous resolution of the disease (Mori *et al*, 1995), and of successful treatment with a combination of cyclosporine and the anti-CD3 monoclonal antibody OKT3 (Yasukawa *et al*, 1994) or antithymocyte globulin and steroids (Prince *et al*, 1991). Nafmostat mesilate, a serine protease inhibitor that inhibits cytotoxic T cells, has been used with transient improvement (Ryo *et al*, 1999). Chloroquine, another serine protease inhibitor that inhibits cytolytic T cells *in vitro*, is being considered as a form of therapy (Nishimura *et al*, 1998). Some of the newer anti-GVHD reagents such as daclizumab, a humanized anti-interleukin 2 receptor alpha chain antibody that has shown promise in the treatment of steroid-resistant acute GVHD after BMT, may be considered in the treatment of this disease (Przepiorka *et al*, 2000). The rapid and fulminant onset of TA-GVHD associated with pancytopenia and resultant overwhelming infections contribute to the high mortality seen in TA-GVHD (Brubaker, 1986).

PREVENTION

Prevention of TA-GVHD is of paramount importance as it cannot be treated successfully. Patients at risk must be identified and transfused with irradiated cellular blood products, as irradiation inhibits proliferation of donor lymphocytes and thereby their initiation of GVHD.

Irradiation

The irradiation dose must be chosen such that there is no significant adverse effect upon red cell, platelet or granulocyte function, while at the same time abrogating the responsiveness of the lymphocytes. Various tests have been used to determine the optimum radiation dose required to inhibit lymphocyte proliferation. These include the *in vitro* response of lymphocytes to mitogens, to allogeneic cells in a mixed leucocyte culture (MLC), and the use of the limiting dilution assay (LDA). At a dose of 1500 cGy, 90% of mitogen response is inhibited, while at 5000 cGy mitogen response is decreased by 97% (Valerius *et al*, 1981). Therefore, at 5000 cGy about 3% of lymphocytes survive irradiation and are capable of a response to mitogens. However, allogeneic cells are far more radiosensitive. As little as 500 cGy can abolish the response of lymphocytes in a MLC (Sprent *et al*, 1974; Leitman & Holland, 1985). One of the limitations of the MLC in determining optimum irradiation dosage is that the MLC detects only a 1–2 log decrease (90–99%) in functional T cells and is therefore not a reliable test to detect low numbers of immunocompetent T cells. A decrease in T cells of > 2 log is needed to prevent GVHD. Studies using the LDA, which is more sensitive than the MLC, have shown that irradiation doses of 2500–3000 cGy completely inhibit T-cell proliferation (Pelszynski *et al*, 1994). The dose of 2500 cGy has therefore been recommended in the US by the Federal Drug Administration (FDA) as the requirement for irradiation of cellular blood products (Menitove, 1999).

The effects of irradiation on the different constituents of blood have been studied to ensure efficacy and safety of the product after exposure to the optimal dose of irradiation to prevent TA-GVHD (Holland, 1989). Overall, the clinical efficacy of red cells, platelets and granulocytes does not seem to be significantly affected by doses up to 5000 cGy (Button *et al*, 1981; Valerius *et al*, 1981). However, certain significant changes have been noted at doses of 3000–3500 cGy.

Red cell survival is decreased in blood irradiated on d 0 with 3000 cGy and stored for 42 d; the mean 24-h recovery of red cells stored in an additive preservative solution (AS-1, Adsol, Baxter Healthcare, Deerfield, IL, USA) is 68% versus 78% in non-irradiated stored blood (Davey *et al*, 1992). Irradiation affects the red cell membrane with an increased loss of potassium from the cell and the plasma haemoglobin increases significantly by 35 d of storage of an irradiated product (Ramirez *et al*, 1987; Moroff *et al*, 1999). The concentration of potassium in stored red cells ranges between 55 and 100 mmol/l. The safety of large volume transfusions to neonates of such stored irradiated products with their high potassium concentration has not been proven (Strauss, 2000).

These effects of irradiation on cellular products have resulted in the recommendation by the American Association of Blood Banks (AABB) that red cells cannot be stored for longer than 28 d after irradiation, with the total storage time not exceeding that for non-irradiated red cells. Red cells for intrauterine, neonatal or paediatric transfusion or for exchange transfusion should be irradiated immediately prior to use (Menitove, 1999).

At a dose of 5000 cGy, the reported effects on both platelet function and survival have been variable. In some studies, platelet function has been reported to remain unchanged, while others report significant deleterious effects. These effects include a decrease in expected platelet increment post transfusion by one-third, decreased aggregation in response to collagen and a decreased ability to correct the aspirin-induced bleeding time (Holland, 1989). At doses of 2500–3500 cGy, platelet recovery and survival are within the normal range (Read *et al*, 1988; Duguid *et al*, 1991).

Similarly, the effects of irradiation on granulocyte function are variable. Although low doses of irradiation (500 cGy) mildly decrease chemotactic function, this effect becomes clinically significant only at doses greater than 10 000 cGy (Sprent *et al*, 1974). Phagocytosis and bactericidal function are only affected at irradiation doses greater than 40 000 cGy (Button *et al*, 1981; Valerius *et al*, 1981).

The recommendations for irradiation of cellular blood products for Britain, the United States and Japan are outlined in Table II. The British Council for Standards in Haematology (BCSH) guidelines recommend a minimum dose of 2500 cGy, with no part of the product receiving > 5000 cGy (BCSH Blood Transfusion Task Force, 1996). The AABB recommends a minimum dose of 2500 cGy at the centre of the irradiation field with a minimum dose of 1500 cGy at any point in the field (Menitove, 1999), while the Japanese guidelines recommend a dose between 1500 and 5000 cGy (Asai *et al*, 2000). Any of these recommended doses, if correctly delivered, should be adequate to prevent TA-GVHD.

The dosage, the equipment and the conditions of irradiation must be verified to ensure that the irradiator is functioning properly. Dose mapping should be performed annually. There should be a method of confirming that irradiation has taken place, such as the use of a radiation sensitive label which changes visibly when exposed to a defined dose of irradiation (Moroff *et al*, 1997). Although irradiation is currently the best method to minimize the risk of developing TA-GVHD in patients at risk, failures have been reported. These include the development of TA-GVHD in three patients transfused with irradiated blood, two at doses of 2000 cGy and one at 1500 cGy (Drobyski *et al*, 1989; Sproul *et al*, 1992; Lowenthal *et al*, 1993). It is not known, however, whether these cases represent process failures.

Indications for irradiation

The indications for irradiation have changed with the increased awareness and reports of TA-GVHD. The AABB Standards recommend irradiation for patients at risk for TA-GVHD (to be defined by the transfusion service), intrauterine transfusions, directed donations from family members and donations from an individual selected for HLA compatibility (Menitove, 1999). The British standards are similar (BCSH Blood Transfusion Task Force, 1996). In neither country is irradiation of blood products mandatory for patients undergoing cardiac surgery or for preterm infants.

The recently published guidelines for irradiation of blood and blood products from Japan reflect the different risk factors in that country (Asai *et al*, 2000). Their indications for irradiation are much broader; they recommended transfusion of irradiated blood for cardiovascular (CV) surgery as early as 1992. By 1995, they had expanded their recommendations for the use of irradiated blood to patients undergoing cancer surgery. They also recommended irradiation of all fresh blood, defined as blood < 72 h old. Their most recent set of guidelines extends their recommendations still further and includes older recipients (> 65 years old), patients with massive blood loss or severe trauma. Blood stored for up to 14 d should also be irradiated. They now state that 'transfusion of blood from relatives should be avoided', a big change from their practice less than 10 years ago (Ohto & Anderson, 1996a,b).

Effectiveness of irradiation

The effectiveness of the introduction of these preventative measures is best shown in data from Japan. Juji *et al* (1989) surveyed 340 hospitals in Japan, reviewing the cardiac surgical cases between 1981 and 1986. Over 60 000 surgical procedures were carried out; 96 patients developed TA-GVHD, an incidence of 1 in 658 cases or 0·15%. The number of cases continued to increase until 1990, after which there was a slow decline. The reported increase of TA-GVHD may have been related in part to an increased awareness, recognition of the clinical picture and diagnosis. The decreased incidence was attributed to the implementation of measures, including the increased use of irradiation of blood products and of filtration, and a decreased use of fresh blood and directed donations (Hato *et al*, 1994). As a number of changes were introduced at the same time, they could not determine which of these factors was responsible for the decrease of TA-GVHD.

With the introduction of more stringent guidelines there has been a further decrease in TA-GVHD in Japan, with the number of annual cases reported ranging between 9 and 14

Table II. Comparison of radiation guidelines, including methods and indications.

	Britain[1]	US[2]	Japan[3]
Techniques	Irradiation	Irradiation	Irradiation
Dose	Minimum −2500 cGy No part > 5000 cGy	2500 cGy at centre of product Minimum 1500 cGy at any point	Between 1500 cGy and 5000 cGy
Type of product	All cellular products: Whole blood RBCs Platelets Granulocytes	All cellular products: Whole blood RBCs Platelets Granulocytes	All cellular products: Whole blood RBCs Platelets Granulocytes Fresh plasma
Age of product	RBCs < 14 d after collection Platelets – any time during 5 d storage For exchange or intrauterine transfusion: < 24 h	RBCs – any time Platelets Granulocytes	RBCs: ≤ 3 d – regardless of recipient ≤ 14 d – if clinically indicated at any time – if patient immunocompromised
Expiration	RBCs stored 14 d after irradiation	RBCs stored up to 28 d after irradiation or original outdate, whichever is sooner	Irradiated RBCs – up to 3 weeks after collection
General	All blood from relatives All HLA selected products All granulocytes	All blood from relatives All HLA selected products	All blood from relatives All HLA selected products
Neonates	Intrauterine transfusions (IUT) exchange transfusions in IUT babies	Intrauterine transfusions	Intrauterine and exchange transfusions
'Top-up' transfusion	IUT babies	*	Pre-term
SCID	All	All	All
BMT – Allogeneic	All – at least 6 months post BMT; longer in selected patients	All	All
Autologous	All – at least 3 months post BMT; 6 months if TBI used		
Leukaemia	No	*	To be considered
Hodgkin's disease	All stages	*	To be considered
Purine analogues	All	*	Not discussed
Non-Hodgkin's lymphoma	Not necessary – under review	*	To be considered
Solid tumours	No	*	To be considered
Solid organ transplants	No	*	To be considered
Aged > 65 years	Not discussed	No	Yes
Massive blood loss	Not discussed	No	Yes
CV surgery	No	No	Yes
AIDS	No	No	No

*According to policies and procedures developed by the blood bank or transfusion service.

1. BCSH Blood Transfusion Task Force (1996).
2. Menitove (1999).
3. Asai *et al* (2000).

from 1993 to 1997. In the last 4 years, the number of cases has decreased to two in 1998, four in 1999 and none in 2000 or 2001. Approximately 18 million cellular blood products are provided to hospitals of which more than 95% are irradiated prior to use (T. Juji, personal communication). These results show that TA-GVHD can be prevented.

Leucocyte depletion

Leucocyte depletion alone cannot be used as the sole method to prevent TA-GVHD in a patient at risk as the minimum number of cells required to cause this disease is unknown, and the cells remaining in the blood product are viable.

Pre-storage leucocyte depletion of blood products, that is filtration of the blood at the time of collection, is in routine use in many countries, including Britain and Canada. All leucocyte-depleted red cells issued for transfusion should contain $< 5 \times 10^6$ viable white blood cells in the final component; however, a small percentage of units may contain more leucocytes (Kao *et al*, 1995). A dose of lymphocytes as low as 1×10^4/kg has been reported to cause GVHD in an immunocompromised host (Rubinstein

et al, 1973). Also, TA-GVHD has been reported after the transfusion of leucocyte-depleted products (Akahoshi *et al*, 1992; Hayashi *et al*, 1993). As with irradiation, there may always be a technical failure, such as a problem with the filter itself and/or human error, that may result in products being released that contain more white cells than the accepted standard.

The true effect of prestorage leucocyte depletion on the development of TA-GVHD will never be quantified as other preventative measures have already been put in place.

A combination of leucocyte depletion and irradiation of products given to patients at risk should provide additional safety and ultimately result in the prevention of TA-GVHD. Prevention can only be effective if all patients at risk of developing TA-GVHD are identified and provided with an irradiated product.

PATHOGEN INACTIVATION

Recent studies have looked at a variety of methods of preventing the transmission of infectious agents, such as bacteria and protozoa, which are transmitted in cells, mainly the monocytes and lymphocytes (Council of Europe Expert Committee in Blood Transfusion, 2001). The methods under investigation include photoinactivation, with the use of the psoralen S59 with ultraviolet A light, or the use of photoactive phenothiazine dyes. These methods render T lymphocytes inactive and incapable of proliferating and causing TA-GVHD. Such methods also inactivate viruses and bacteria and thereby sterilize the product. These procedures, designed primarily to inactivate pathogens, are still in development. Numerous logistical problems will have to be addressed, not the least of which is the cost of introducing any of these methods into routine use. However, if any method that inhibits lymphocyte proliferation becomes part of routine practice, the risk of TA-GVHD would be minimized further. Whether or not these would replace irradiation remains to be seen, as the problem of human error and process failures will not be eliminated.

SUMMARY

TA-GVHD can be prevented, but only if the appropriate safeguards are in place. A method that inhibits proliferation of lymphocytes in cellular products must be used. At present, irradiation is the only proven method. The clinician must be aware of this syndrome and of patients at risk and must request irradiated products as clinically indicated. The Transfusion Medicine Department must have appropriate policies in place that are consistent with the standards of the country and take into account risk factors for the specific population in question. Unfortunately, TA-GVHD will not be totally eliminated. Process and management failures will remain. Education about and awareness of this disease are of crucial importance so that patients at risk are defined and are transfused with irradiated products.

Department of Pediatrics and Child Health, University of Manitoba, Winnipeg MB, Canada MARLIS L. SCHROEDER

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