Invited article

TRANSPLANT International

Transplant Int (1991) 4: 67–71

Graft-versus-host disease in solid organ transplantation

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Received August 20, 1990/Accepted January 3, 1991

Abstract. Graft-versus-host disease is well recognized in bone marrow transplantation, but has only recently been described in solid organ transplantation. Two such cases in liver graft recipients, proven by the demonstration of donor type HLA antigens in the peripheral blood and marrow on tissue typing, are described in this paper. The literature on this subject is reviewed and the treatment discussed. It is postulated that there is an order of risk of development of graft-versus-host disease depending on the amount of viable lymphoid tissue included with the transplanted organ as follows: small bowel > heart-lung > liver > kidney > heart. It seems likely that this condition has been substantially underdiagnosed in the past and that greater awareness of the possibility of graft-versus-host disease in solid organ recipients will lead to the recognition of further cases and allow appropriate treatment to be promptly instituted.

Key words: Graft-versus-host disease, in liver transplantation – Liver transplantation graft-versus-host disease

Graft-versus-host disease (GVHD) is commonly encountered in bone marrow grafting, but has only recently been reported in solid organ transplantation [2, 6, 8, 16]. Billingham defined the requirements of GVHD as follows [3]:

1. histocompatibility differences between donor and recipient;

2. the presence of immunocompetent cells in the graft; and

3. inability of the host to reject the graft.

These criteria can most clearly be seen to be met in the recently reported fatal GVHD in immunocompetent heterozygous recipients of fresh blood from HLA-homozygous related donors [10, 17]. In cases such as these the antigens on the donor cells will not be recognized as foreign, while the donor cells can still mount an immune response against the histoincompatible antigens in the recipient. GVHD has similarly been encountered following blood transfusions in patients immunocompromised by malignancy and chemotherapeutic agents [5, 11, 18]. The target organs of skin, liver, gut and bone marrow involved in the process show increased expression of both class-I and class-II antigens in association with an infiltrate of donor CD4+ and CD8+ cells [12].

Lymphoid tissue can also be transferred as part of a solid organ graft from a donor to a recipient who will then receive immunosuppressive agents to prevent rejection of the donor organ. We report here two such cases of GVHD in liver transplant recipients.

Materials and methods

Patients

Patient 1. A 57-year-old Japanese male of blood group B+ was referred for transplantation with a diagnosis of hepatoma complicating pre-existing cirrhosis. Nine years prior to referral he had first

Table 1. Reactions of peripheral blood lymphocytes of patient 1 and his donor with anti-HLA A and B sera in the cytotoxic test, before and at varying times after transplantation, to illustrate chimaerism at time of GVHD. Recipient groups, HLA-A10, B12, B40; donor groups, HLA-A11, A28, B14, B27. + +, 80–100% cell death; +, 40–79% cell death; W, 20–39% cell death; -, < 20% cell death

HLA specificity	Donor	Recipient pretransplant	Days post-transplant		
			21	24	31
A10	–	+	+	+	W
A11	+	–	W	+	W
A28	+	–	+	+	+
B12	–	+	+	+	+
B14	+	–	+	+	+
B27	+	–	+	+	+
B40	–	+	+	W	W
Negative control	–	–	–	–	–

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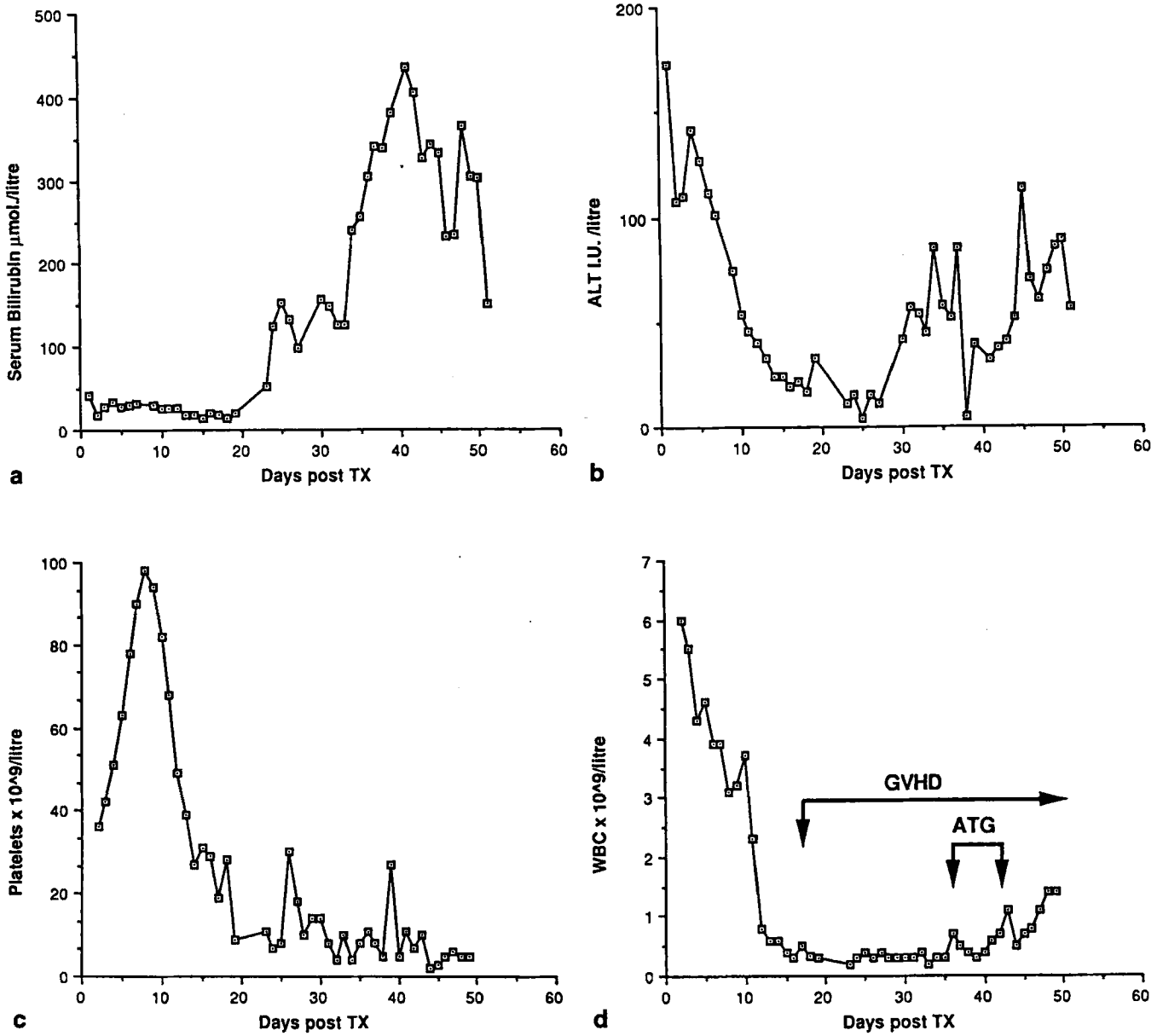


Fig. 1 a–d. Biochemical and haematological course of patient 1, who died on day 51

been found to have abnormal liver function tests and was noted to have an alcohol intake of 100 g/day. He stopped drinking but, nonetheless, soon became symptomatic. Over the ensuing years he received treatment with several courses of azathioprine and steroids on a presumptive diagnosis of chronic active hepatitis prior to eventual diagnosis of hepatoma. The donor was a 22-year-old male of blood group B – who had died as the result of a head injury. The preservation time was 600 min. The operation was uneventful and the total blood loss was 12.4 l, which was replaced by transfusion of banked blood, fresh frozen plasma and platelets.

Initial progress following transplantation was excellent with near normal liver function. Immunosuppression was with a triple regime of prednisolone 1 mg/kg, azathioprine 1.5 mg/kg and cyclosporin A (CyA). Neutropenia and pyrexia developed on the tenth postoperative day, and a rash developed over the ensuing days. A skin biopsy performed on the 18th postoperative day was compatible with GVHD, and treatment commenced with CyA 4 mg/kg i.v. and high-dose methyl prednisolone commencing at 10 mg/kg tapering down at 4-day intervals. Tissue typing studies carried out on peripheral blood lymphocytes between days 21 and 31 showed both donor and recipient HLA antigens present in roughly equal proportions (Table 1). The rash largely resolved on increased immunosuppression but diarrhoea and a marked bleeding tendency continued. Bone marrow aspirates showed an acellular picture and pancytopenia persisted, requiring support with regular transfusion of blood and blood products. The patient's marrow showed no signs of recovery and a late course of rabbit antithymocyte globulin (ATG) was without effect. He died on the 51st postoperative day. His biochemical and haematological course are summarized in Fig. 1.

Patient 2. A 55-year-old Caucasian male of blood group A underwent transplantation for hepatoma complicating pre-existing cryptogenic cirrhosis. The donor was a 22-year-old male of blood group A who had died as the result of a head injury, and the preservation time was 889 min. The operation was uneventful, and total blood loss was 7 l, which was replaced by transfusion of banked blood, fresh frozen plasma and platelets.

Initial progress was excellent. Immunosuppression was with a triple regime of prednisolone 1 mg/kg, azathioprine 1 mg/kg and CyA, combined with an initial 10-day course of Campath 6, an IL2-R blocker. On the 19th postoperative day he developed a mild rash, but remained well. Tissue typing studies on peripheral blood lympho-

Table 2. Reactions of peripheral blood lymphocytes of patient 2 and his donor with anit-HLA A and B sera in the cytotoxic test, before and at varying times after transplantation, to illustrate origin of circulating blood lymphocytes at time of GVHD. Recipient groups, HLA-A11, A28, B35; donor groups, HLA-A1, A11, B8, B37. Symbols as in Table 1

HLA specificity	Donor	Recipient pretransplant	Days post-transplant					
			19	29	31	37	38	43
A1	+	−	+	+	+	W	−	−
A11	+	+	+	+	+	+	+	+
A28	−	+	+	−	−	+	+	+
B8	+	−	−	+	+	W	−	−
B35	−	+	+	−	−	+	+	+
B37	+	−	−	+	+	−	−	−
Negative control	−	−	−	−	−	−	−	−

cytes showed principally recipient antigens but also some evidence for the presence of donor HLA antigens (Table 2). On day 30 the patient developed a severe rash (Fig. 2), diarrhoea and neutropenia. Skin biopsy was compatible with GVHD, and tissue typing studies on peripheral blood lymphocytes now showed only donor HLA antigens (Table 2). He was treated with a 7-day course of rabbit ATG, high-dose methyl prednisolone (20 mg/kg per day tapering at 3-day intervals) and oral CyA.

Sequential tissue typing studies showed a progressive return of recipient-type antigens which had reverted fully to recipient type in both blood and bone marrow by day 38. Liver function remained excellent throughout, without evidence of rejection. His recovery was complicated by a streptococcal septicaemia, Herpes simplex infection and CMV pneumonitis and hepatitis requiring treatment with ganciclovir. He made a satisfactory recovery and was discharged home well. He has subsequently been noted to have evidence of recurrent tumour on bone scan 9 months after transplantation. His biochemical and haematological course is summarized in Fig. 3; the late rise in transaminase levels corresponds to the CMV hepatitis.

Technique of tissue typing

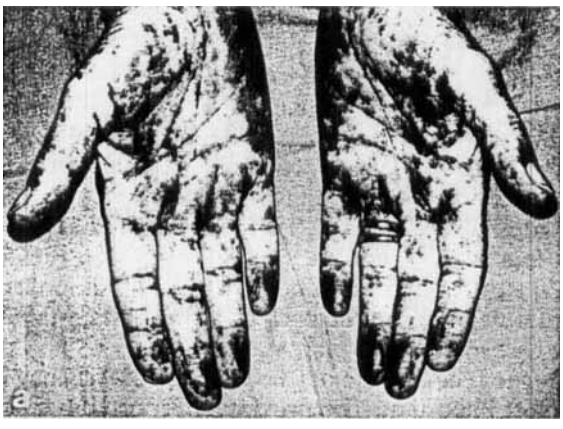
Tissue typing was carried out by the microlymphocytotoxic test using well-authenticated anti-HLA-A and -B sera obtained from the UK Transplant Service. Tests were performed in duplicate. One set was stained with eosin and read by phase-contrast microscopy and the other stained with ethidium bromide and acridine orange and read by fluorescence microscopy. The percentage cell death was assessed as accurately as possible by two independent, experienced scientists. The same suite of sera was used to test cryogenically stored lymphocytes of the donor and the recipient pretransplant, and at various times after transplant fresh lymphocytes were tested to monitor GVHD. For each anti-HLA test serum reacting with antigens of the donor or the recipient, comparison of the amount of cell death pre- and post-transplant indicated fluctuations in the proportion of donor and recipient cells circulating in the recipient.

Discussion

GVHD is a major cause of mortality and morbidity in bone marrow transplant recipients [4]. Little lymphoid tissue is transferred to the recipient in cardiac transplantation, and no cases of GVHD have yet been reported in such patients. However, other solid organ grafts involve the transfer of variable amounts of lymphoid tissue, a situation potentially exacerbated by improved preservation techniques [9] and resulting in the transfer of greater quantities of viable lymphoid tissue.

The earliest clearcut cases of GVHD in human solid organ recipients were reported in pancreas transplant recipients who were given composite grafts including the spleen [16]. This technique has been abandoned, and GVHD has not been reported in patients receiving a pancreas alone. A transient haemolytic anaemia has been observed 12 to 21 days after transplantation in recipients of ABO-compatible liver grafts of different ABO type (e.g. O to A, B or AB), but this process is usually mild and self limiting [13].

A similar haemolytic anaemia has been reported from our own unit in two renal transplant recipients who also received ABO-compatible but non-identical grafts [1]. This haemolysis has been attributed to anti-host isoagglutinin production by immunocompetent cells within the graft. More recently, a single case of severe GVHD in a liver recipient has been reported with a successful outcome after treatment with high-dose steroids and equine ATG [6]. An interesting feature of this case, and the



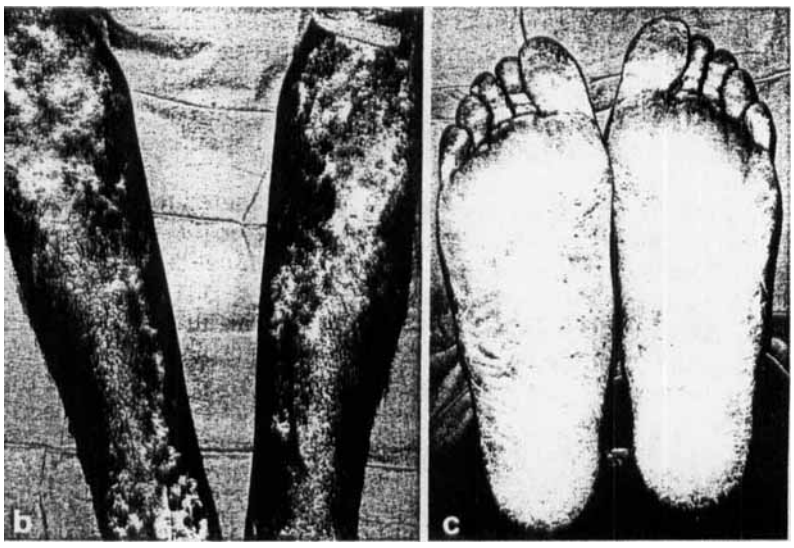


Fig. 2a–c. The rash of GVHD seen in patient 2

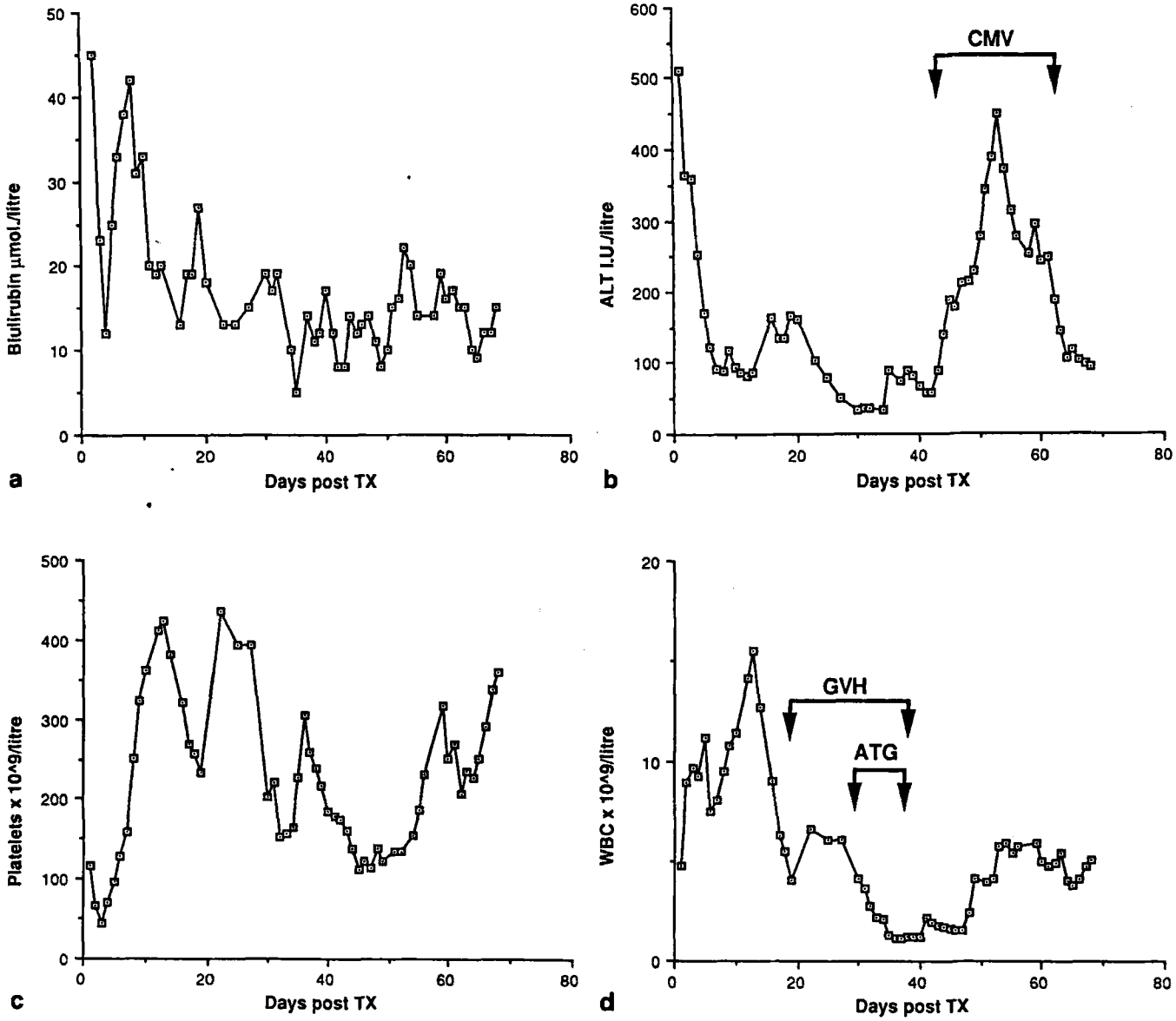


Fig. 3 a–d. Biochemical and haematological course of patient 2

cases presented here, is the maintenance of good liver function at the time of onset of GVHD, the liver being unaffected by the transplanted lymphoid tissue which would recognize it as 'self'. The late abnormalities in liver function tests in our two cases can be attributed to haemolysis and sepsis in patient 1 and CMV hepatitis in patient 2.

The risk of developing GVHD may be attributed to a number of factors relating to either the graft or the recipient. Dealing with the graft first, we may postulate that the risk will increase with increasing amounts of lymphoid tissue being transferred. An order of risk can thus be suggested depending on the organ being transplanted as follows: heart < kidney < liver < heart-lung < small bowel.

The high risk of GVHD in small-bowel transplantation has been well recognized in experimental models, and surgical excision of lymph nodes from such grafts or donor pretreatment with monoclonal antibodies have both been shown to be effective in reducing the incidence [8, 14]. In the case of the recipient of a combined liver and small-bowel graft, OKT3 was administered both to donor and subsequently to the recipient for 14 days. A mild episode of GVHD did occur, but the patient has made a satisfactory recovery [8]. On the 14th postoperative day the peripheral blood contained 38% lymphocytes of donor type and this percentage decreased to less than 2% by day 21 without additional treatment. The occurrence of GVHD in heart-lung transplants has also recently been recognized [19] although it seems likely that this condition has been underdiagnosed in the past, as has almost certainly been the case in liver transplantation.

Turning to recipient factors, host immunodeficiency is clearly most important. The previously reported case of GVHD in a liver transplant recipient occurred in a patient with alcoholic cirrhosis and preoperative lymphocytopenia, and the two cases reported here were both tumour patients, one of whom had, in addition, previously re-

ceived immunosuppressive treatment. It is interesting to speculate on the role of graft/donor histocompatability. Presumably the same risks of developing GVHD apply to living related liver grafts [7, 15] as apply to HLA-homozygous blood transfusions from relatives [17] and a rate of 1 in 650 cases as observed in Japan [10] might be expected. Whether or not closer HLA matching has any bearing on the liability of an individual to develop GVHD must await further reports.

GVHD occurring in a solid organ recipient must be considered in the differential diagnosis of pancytopenia, usually attributed to azathioprine or CMV infection, or of a rash, which will usually be attributed to a drug reaction or viral infection.

GVHD will sometimes present serologically as a mixture of donor and recipient HLA antigens demonstrable on the 'recipient' peripheral blood lymphocytes, as in patient 1, and the diagnosis may be confirmed by tissue typing studies on peripheral blood lymphocytes showing the presence of donor-incompatible antigens. However, there may be a complete 'take-over' of the recipient peripheral blood lymphocytes by cells of donor origin as occurred on days 29–31 in the case of patient 2, and therefore it is essential to know both donor and recipient HLA groups before transplantation. The prognosis is poor when the GVHD is sufficiently severe to cause marrow failure. This was the case in our first patient, and was reflected by the low circulating platelet and granulocyte counts. If a high index of supicion is maintained and the condition is recognized early, appropriate treatment can be instituted promptly and the chances of a satisfactory response increased.

References

1. Bevan PC, Seaman M, Tolliday B, Chalmers DG (1985) ABO haemolytic anaemia in transplanted patients. Vox Sang 49: 42–48
2. Bhaduri BR, Tan KC, Humphreys S, Williams R, Donaldson P, Vergani D, Mowat AP, Mieli-Vergani G (1991) Graft versus host disease after orthotopic liver transplantation in a child. Transplant Proc (in press)
3. Billingham RE (1966) The biology of graft-versus-host reactions. Harvey Lect 62: 21–78
4. Bortin MM (1987) Acute graft-versus-host disease following bone marrow transplantation in humans: prognostic factors. Transplant Proc 19: 2655–2657
5. Brubaker DB (1986) Transfusion-associated graft-versus-host disease. Hum Pathol 17: 1085–1088
6. Burdick JF, Vogelsang GB, Smith WJ, Farmer ER, Bias WB, Kaufmann SH, Horn J, Colombani PM, Pitt HA, Perler BA, Merritt WT, Melville Williams G, Boitnott JK, Franklin Herlong H (1988) Severe graft-versus-host disease in a liver-transplant recipient. N Engl J Med 318: 689–691
7. Cotton P (1990) Living donor liver transplants cap surgical research for decade of 1980's. JAMA 263: 13–14
8. Grant D, Wall W, Mimeault R, Zhong R, Ghent C, Garcia B, Stiller C, Duff J (1990) Successful small-bowel/liver transplantation Lancet II: 181–184
9. Jamieson NV, Sundberg R, Lindell S, Claesson K, Moen J, Vreugdenhil PK, Wight DGD, Southard JH, Belzer FO (1988) Preservation of the canine liver for 24–48 hours using simple cold storage with UW solution. Transplantation 46: 517–522
10. Juji T, Takahashi K, Shibata Y, Ide H, Sakakibara T, Ino T, Mori S (1989) Post transfusion graft-versus-host disease in immunocompetent patients after cardiac surgery in Japan. N Engl J Med 321: 56
11. Kessinger A, Armitage JO, Klassen LW, Thaler M, Shamiss A, Orgad S, Huszar M, Nussinovitch N, Meisel S, Gazit E, Lavee J, Smolinsky A (1987) Graft-versus-host disease following transfusion of normal blood products to patients with malignancies. J Surg Oncol 36: 206–209
12. Nakhleh RE, Snover DC, Weisdorf S, Platt JL (1989) Immunopathology of graft-versus-host disease in the upper gastrointestinal tract. Transplantation 48: 61–65
13. Ramsey G, Nusbacher J, Starzl TE, Lindsay GD (1984) Isohemagglutinins of graft origin after ABO-unmatched liver transplantation. N Engl J Med 311: 1167–1170
14. Schraut WH (1988) Current status of small-bowel transplantation. Gastroenterology 94: 525–538
15. Singer PA, Siegler M, Whitington PF, Lantos JD, Emond JC, Thistlethwaite JR, Broelsch C (1989) Ethics of liver transplantation with living donors. N Engl J Med 321: 620–622
16. Starzl TE, Iwatsuki S, Shaw BW Jr, Green DA, Thiel DM van, Nalesvile MA, Nusbacher J, Diliz-Pere H, Hakala TR (1984) Pancreaticoduodenal transplantation in humans. Surg Gynecol Obstet 159: 265–272
17. Thaler M, Shamiss A, Orgad S, Huszar M, Nussinovitch N, Meisel S, Gazit E, Lavee J, Smolinsky A (1989) The role of blood from HLA-homozygous donors in fetal transfusion-associated graft-versus-host disease after open-heart surgery N Engl J Med 321: 25–28
18. von Fliedner V, Higby DJ, Kim U (1982) Graft-versus-host reaction following blood product transfusion. Am J Med 72: 951–961
19. Wood H, Higenbottam T, Joysey V, Wallwork J (1990) Graft-versus-host disease after human heart-lung transplantation (abstract). XIII International Congress of the Transplantation Society, San Francisco 1990