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Original Contribution

REVERSAL OF GRAFT-VERSUS-HOST DISEASE WITH INFUSION OF AUTOLOGOUS BONE MARROW

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☐ **Abstract – Graft-versus-host disease (GVHD) remains a major complication of bone marrow transplantation. This report describes reversal of GVHD by infusion of stored recipient bone marrow following combined liver-bone marrow allotransplantation. Graft-versus-host disease developed at the end of the first postoperative week. The skin involvement progressively spread to approximately 80% of the body surface and was not affected by modification of the immunosuppressive treatment. On the 42nd and 43rd postoperative day 1.23×10^8 and 1.6×10^8 autologous bone marrow cells per kg of recipient body weight were infused. The skin rush began to dramatically improve and resolved within 2 wk from the autologous marrow infusion. Autologous bone marrow storage previous to allogeneic bone marrow transplantation for tolerance induction could constitute a safety net in case of occurrence of GVHD.**

☐ **Keywords – Transplantation; Bone marrow, Human; Graft-versus-host.**

The concept of graft-versus-host disease (GVHD) as the recognition of the host by immunologically competent cells of the graft was first delineated by Billingham and Brent (1), following spleen cell and bone marrow transplantation. However, it was soon recognized that the same problem could affect recipients of organs that are rich in lymphoid tissue (2–12). In this report, we describe reversal of GVHD by infusion of stored autologous bone marrow in a recipient of a combined liver-bone marrow transplant.

CASE REPORT

A 56-yr-old man underwent upper abdominal exenteration and liver transplantation on July 16, 1992 for a gastric leiomyosarcoma with liver metastases. Using standard techniques autologous bone marrow cells were harvested before the surgical procedure and stored in liquid nitrogen (14). A few hours before operation, the patient was treated with a single dose of 550 rads thoraco-abdominal lymphoid irradiation (TLI). Bone marrow cells from the same donor as the liver allograft were separated from vertebral bodies obtained at the time of the multiorgan donor procedure. Then, 2 × 108 bone marrow cells per kg of recipient body weight were infused IV through a central line immediately after the orthotopic liver transplant. Postoperative immunosuppression was with conventional doses of FK 506 and prednisone (Fig. 1).

A skin rash developed at the end of the first postoperative week that was initially mild and confined to the areas exposed to the preoperation irradiation. The rash worsened during the second postoperative week, and on the 15th postoperative day (POD), the diagnosis of GVHD that was Grade 2 by the criteria of Lerner et al. (13) was made on a skin biopsy. On days 21 and 32 progressively more florid changes compatible with acute GVHD were seen in second and third skin biopsies. The skin biopsy obtained on POD 15 showed a mild, predominantly *T*-lymphocyte infiltrate localized to the upper dermis and associated with focal exocytosis and spongiosis. Occasional keratinocyte necrosis was present. Immunoperoxidase studies disclosed that the infiltrating cells were of donor HLA type. The second biopsy on POD 21 revealed more florid changes which included acantholysis and focal cleft formation

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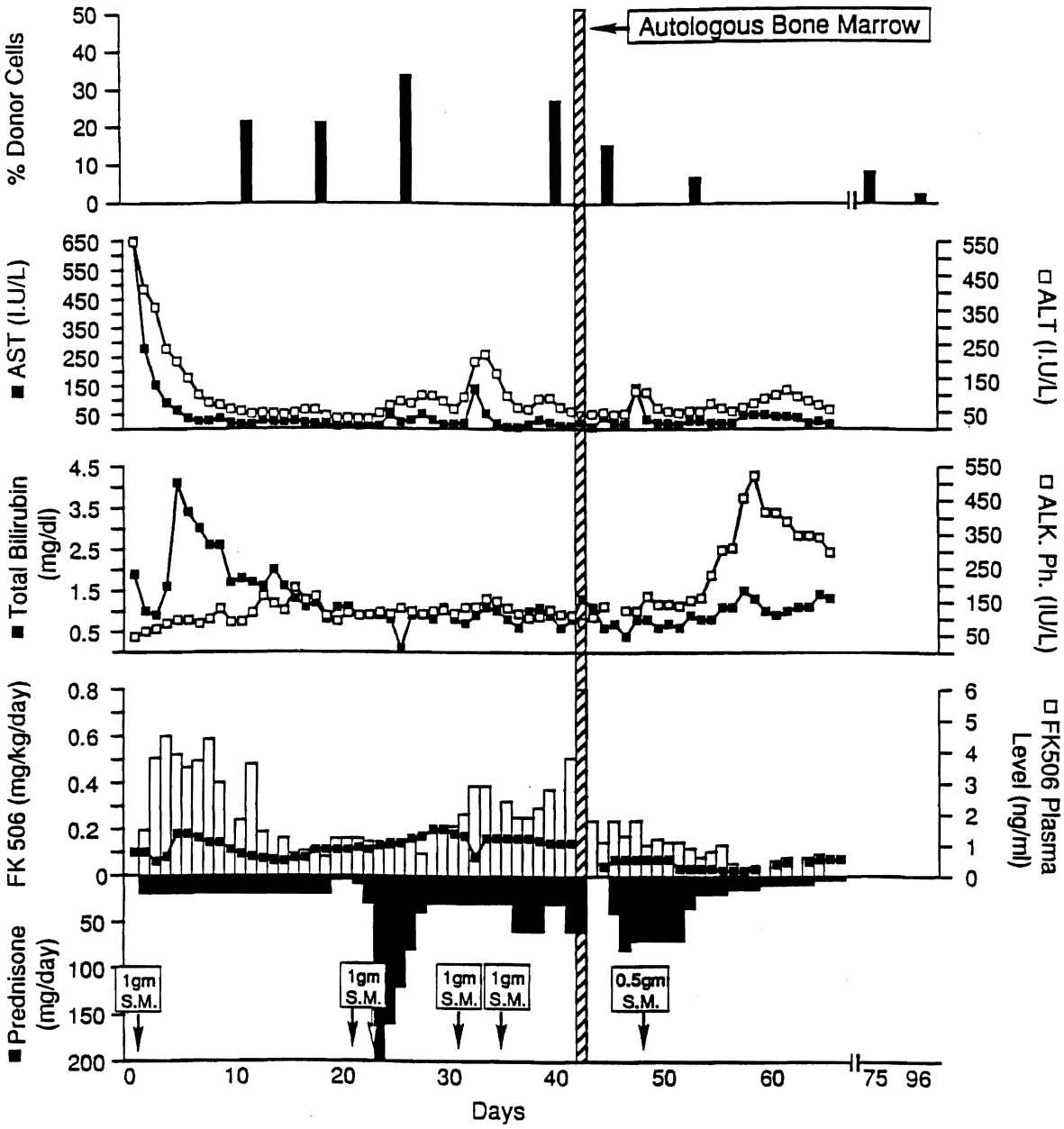
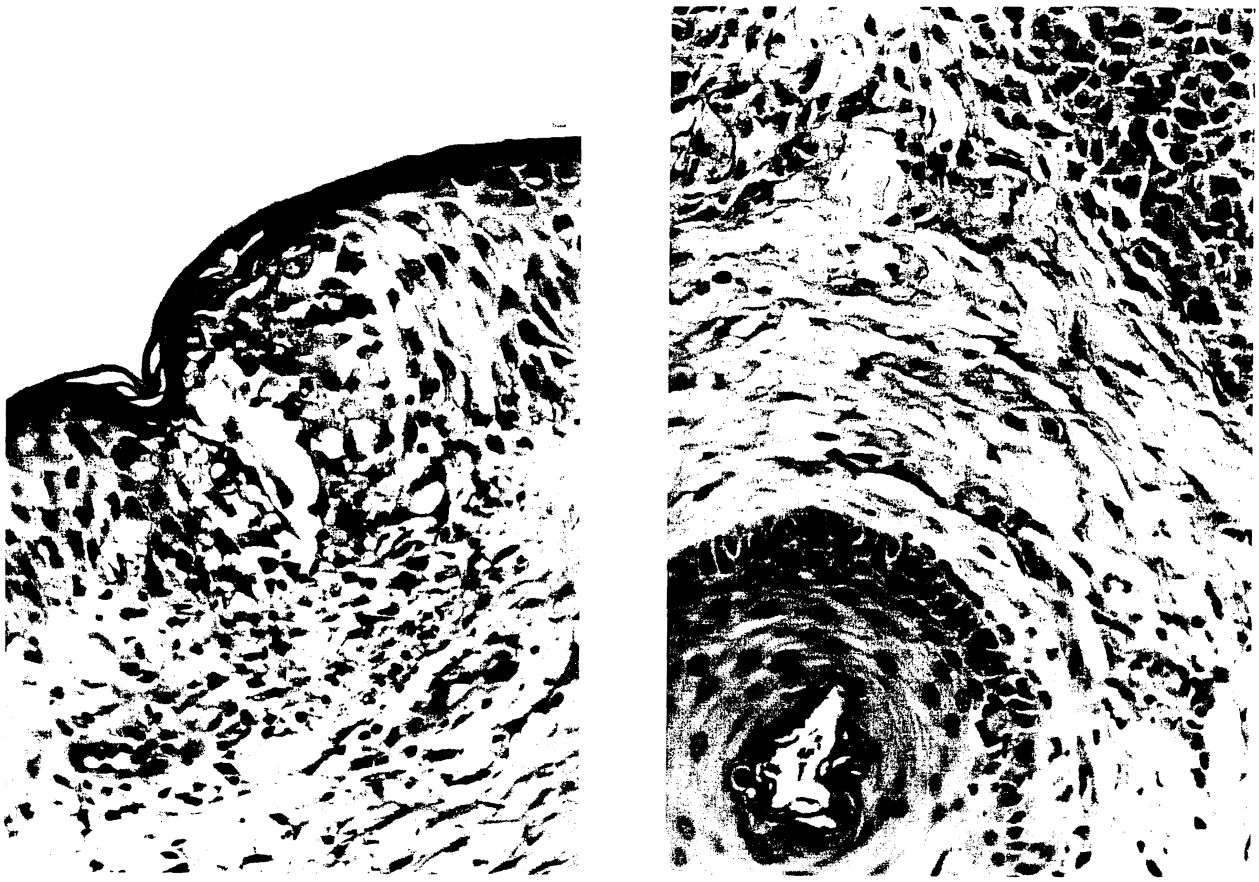


Fig. 1. Clinical course of the combined bone marrow-liver allograft recipient (AST = aspartate aminotransferase; ALT = alanine aminotransferase).

(Fig. 2a), compatible with Grade 3 acute GVHD according to the criteria of Lerner et al. (13). The biopsy on POD 32 showed similar changes with continued damage to keratinocytes and adnexal cells (Fig. 2b) and a significantly more conspicuous inflammatory infiltrate of the upper dermis than previously. Immunoperoxidase stains showed the cells to be *T*-lymphocytes of donor origin. Stains for Epstein-Barr virus were negative. Grossly, the skin involvement spread to approximately 80% of the body surface, including the palms, soles, and face. Its progress was not altered by increases or decreases of FK 506 or prednisone. Bouts of eosinophilia (2,040/mm^3) and diarrhea were recorded. On the 42nd and 43rd POD posttransplantation, 1.23 × 108 and 1.6 × 108 autologous BMC/kg were infused. The skin rash began to dramatically improve 1 wk after the autologous cell infusion and had largely resolved after 2 wk. At skin biopsy, four days after the autologous marrow infusion (POD 47), the donor cell infiltrate was less. Focal exocytosis of mononuclear



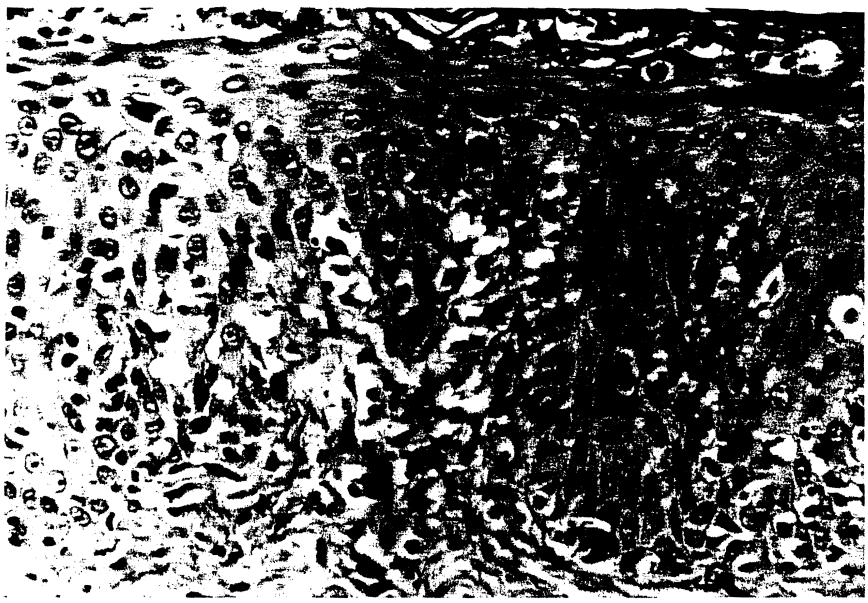
(a) (b)

Fig. 2. (a) Skin biopsy obtained on POD 21. A mononuclear infiltrate of the superficial dermis and epidermis is associated with spongiosis, acantholysis and an area of confluent keratinocyte necrosis. Disruption of the dermal-epidermal junction is also apparent (×250). (b) Skin biopsy on postoperative day (POD) 32 showing an area of dense monoculear inflammation (lower left corner) with involvement and single cell necrosis of hair follicle (upper right corner).

cells was found in association with ballooning of basal cells and aggregates of necrotic keratinocytes in the upper layers of the epidermis (Fig. 2c). Seven days after this (POD 54), skin biopsies had less evidence of acute GVHD ranging from Grade 0 to 2 (Fig. 2d). By this time, the rash caused by GVHD had largely resolved. No additional biopsies were obtained.

The patient, who had been seriously ill, was restored to a sense of well being and was discharged. However, on October 13 (90 POD), he was readmitted with jaundice. A needle biopsy of the liver was diagnosed as acute viral hepatitis. Because of the additional diagnosis of partial biliary obstruction from ampullary dysfunction, the biliary reconstruction of choledochodochostomy was converted to a Roux-y-choledochojejunostomy on October 22, 1992. Another liver biopsy was obtained intraoperatively with which the diagnosis of C-virus hepatitis (HCV) was confirmed by identification of viral RNA using the polymerase chain reaction (PCR). A recipient lymph node and a segment of bowel excised for the biliary reconstruction were taken for immunocytochemical staining and histopathologic staining. After immunocytochemical staining, both the intraabdominal lymph node and segment of jejunum contained sparsely distributed donor cells. This degree of chimerism has been associated with graft acceptance and donor specific nonreactivity (15).

For the 1.5 mo before as well as after the biliary reconstruction, minimum immunosuppression was given with 1 mg FK 506, every other day and 5 mg/day prednisone. Alpha-interferon therapy was started on October 27. The patient was discharged on November 20. On February 24, 1993, when the bilirubin was 1.2 mg%, immunosuppression with FK 506 was discontinued with continuation of 2.5 mg prednisone. On May 4, 1993, he was admitted with a bilirubin of 1.9 mg%



(c)

Fig. 2(c). Inflammation and epidermal damage is still present in the biopsy on postoperative day (POD) 47 (four days after autologous bone marrow infusion) (×250).

which rose over the next 13 days to 9.7 mgm%. Retransplantation was performed on May 17, 1993, but the patient died 4 mo later after repeated intraabdominal and systemic infections. Histopathologically, the primary allograft had findings of hepatitis but there also was evidence of rejection with obliterative arteriopathy and diffuse small duct loss.

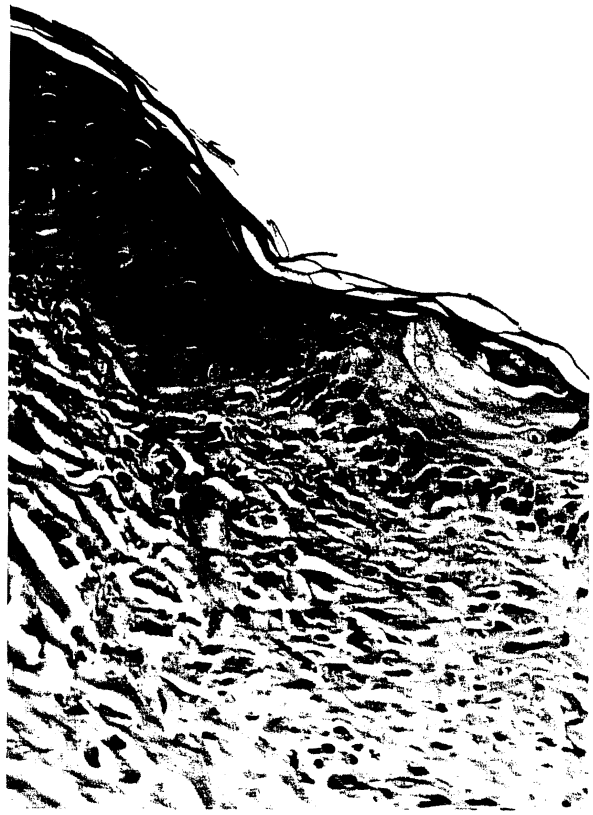
Flow cytometry (FACS II, Becton Dickinson & Co., Mountain View, CA) was used to determine the relative percentages of PBL bearing HLA Class I specific for the donor or recipient. Blood was collected and RBC were ACK-lysed. The remaining cells were stained with anti-HLA Class I monoclonal antibodies (mAbs) (generously provided by Dr. Paul Terasaki) for 30 min at 4°C and counterstained with sandwich when required. Donor cells were detected in the peripheral blood from the time of the first analysis, 11 days following liver transplantation. These accounted for 22–34% of the total cell number out to POD 43 (Fig. 1), but then dramatically decreased following the autologous bone marrow infusion, to 3% at 96 days following transplantation. On POD 227, cytospin examination of a buffy coat preparation stained with donor MHC reactive monoclonal antibodies showed < 1% donor cells. The cytospin during the week before retransplantation was negative for donor cells.

Donor specific proliferation was assessed in MLR pretransplant and monthly intervals postoperatively for 8 mo. Before surgery, the recipient's lymphocytes exhibited significant proliferative responses to both donor and third party HLA unrelated stimulators (Table 1). For the first month postoperatively, the patient's PBL showed no response to either donor or third party. However, the PBL blood sample from day 32 at the time of florid GVHD was stimulated by the stored autologous pre-transplant PBL (3,946 cpm vs. 269 cpm background). Three days after infusion on POD 42–43 of the autologous marrow, the patient's PBL showed highly significant spontaneous prolifer-

Table 1. MLR Responses of Peripheral Blood Lymphocytes from Combined Liver-Bone Marrow Transplant Recipient

		Proliferative Responses (CPM)		
POD	Backg.	Recipient*	Donor*	T.P.*
Pre-Tx	302	167	15,543	10,842
32	269	3,946	173	205
46	32,178	28,058	37,217	41,024
62	1,915	2,415	27,212	11,387
97	1,890	813	42,665	24,362
108	204	225	8,006	5,775
223	975	431	3,994	12,982
227	729	448	8,656	17,723
305	3045	666	10,704	13,730

*Stimulator cells were irradiated 2000R.



(d)

Fig. 2(d). Biopsy on postoperative (POD) 54 from the resolving area of rash shows only a modest mononuclear infiltrate associated with isolated necrotic keratinocytes. Changes of acute GVHD ranged from 0 to 2 in the four samples taken at this time (×250).

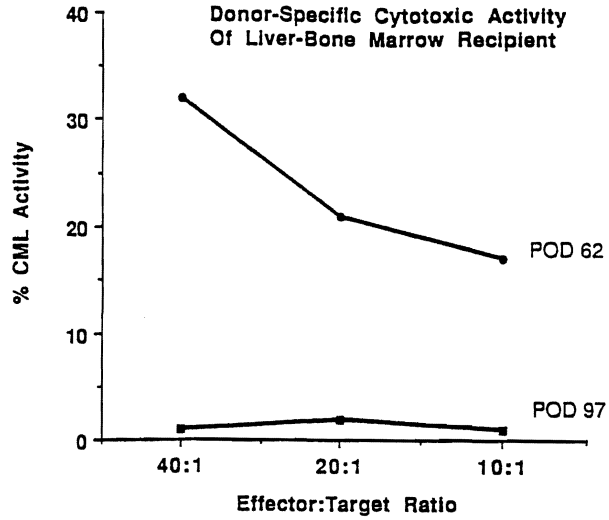


Fig. 3. Donor-specific cytotoxic activity of the combined bone marrow-liver allograft recipient postoperative day (POD).

ation (Table 1) which was not markedly altered by the addition of stored autologous, donor, or recipient cells. However, by POD 62, (20 days after autologous bone marrow infusion) spontaneous proliferation had died down and MLR responses to donor and third party were restored. For the next 2 mo, the patient's proliferative response to donor pretransplant lymphocytes was higher than that to third party. By 7 mo postsurgery, the MLR response to the donor had dropped to only 25% of the response to third party. However, by 10 mo, 6 wk before retransplantation, antidonor party responses had recovered and were similar to those before the primary transplantation.

Significant donor-specific CML activity was observed with the blood sample on POD 62, 20 days following autologous bone-marrow infusion (Fig. 3). Even at a low effector:target ratio (10:1), recipient lymphocytes exhibited 20% donor specific CML activity with no CML reactivity generated towards third party stimulator cells then or later. This donor-specific CML response was not evoked by the donor cells in a subsequent sample on day 97 at which time a significant proliferative response in MLR still was present.

DISCUSSION

These results indicate that stored autologous bone marrow could constitute a safety net for GVHD in splanchnic organ recipients or in clinical trials of bone marrow transplantation that are designed to induce donor specific unresponsiveness for whole organ or non-hematolymphopoietic (i.e., islet) cell transplantation. Neither temporary reduction of immunosuppression nor augmentation of treatment affected the GVHD. After the autologous cell infusion, the skin biopsy was better within 4 days, and dramatic clinical improvement was evident by the end of the week.

Ten days before the bone marrow infusion, the MLR showed no response against donor or third party, indicating the profoundly immunocompromised status of the recipient. This could explain why the recipient peripheral leukocytes were ineffective in control of the GVHD, despite their quantitative advantage relative to the infused bone marrow cells which had been removed and cryopreserved preoperatively, before the patient underwent radiation treatment or was exposed to pharmacologic immunosuppression. The infused autologous cells retained their ability to reverse the GVHD, and ultimately, the alloreactivity of the circulating recipient cells to those of the donor was restored to levels that

were even higher than those observed pre-transplantation. Eventually, the recipient whose immunosuppression had been stopped regained enough immune competence to reject the graft which also was afflicted with severe HCV. The autologous cell treatment could be considered analogous to the "tolerance breaking" experiments described by Billingham, Brent, and Medawar (16).

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