1694

Effects of Preformed Antibodies Induced by Whole Blood Transfusion on Small Bowel Transplantation

S. Fujisaki, N. Murase, A.J. Demetris, M. Tanabe, S. Todo, and T.E. Starzl

ALTHOUGH pretransplant donor-specific transfusions (DST) have been effective in prolonging subsequent allograft survival, alloreactive antibodies are also produced after sensitization with DST. The purpose of this study was to examine the influence of blood transfusion on small bowel transplantation (SBTx).

MATERIALS AND METHODS

Using inbred male LEW ($RT1^l$), BN ($RT1^n$), ACI ($RT1^a$), and PVG ($RT1^c$) rats weighing 200 to 300 g, orthotopic SBTx was performed as described previously.[1] Donor-specific or third-party heparinized whole blood (1.0 mL) was given 7 days before transplantation. As a control, SBTx was performed in untransfused recipients. Furthermore, the effect of blood transfusion on the incidence of graft versus host disease (GVHD) was examined using LEW-to-BN SBTx, in which fatal GVHD occurs under FK506 immunosuppression.[2] BN recipients received either LEW, PVG, whole blood, or no transfusion 7 days before LEW small bowel grafting. FK506 (Fujisawa Pharm Co Ltd, Osaka, Japan) was given intramuscularly at a dose of 0.64 mg/kg/d for 14 postoperative days.

Serum samples for lymphocytotoxic antibody (LAb) assay were obtained from ACI rats 7 days after PVG blood transfusion. To investigate the specificity of the preformed LAbs induced by blood transfusion, the sera were further absorbed with donor (PVG) or syngeneic (ACI) red blood cells (RBCs), which are known to carry surface class I major histocompatibility complex (MHC) antigenic determinants. A complement fixing LAb assay was performed using unfractionated donor or third-party strain lymphocytes as targets according to the method described previously.[3]

In the LEW-to-BN combination, percentages of donor cells in the recipient circulation were examined. Recipient peripheral blood lymphocytes were stained with monoclonal antibody (MAb) 163, which is specific for the $RT1.A^1$ antigen on LEW (kindly provided by Dr H.W. Kunz, University of Pittsburgh, Department of Pathology) and were analyzed using a FACScan flow cytometer.

Statistical analysis was performed using the Mann-Whitney *U* test.

RESULTS

DST significantly ($P < .05$) prolonged the survival of PVG recipients of ACI grafts (median survival 14.0 days, n = 12), LEW recipients of ACI grafts (median survival 16.5 days, n = 6), and BN recipients of LEW grafts (median survival 25.5 days, n = 6) when compared with those without DST (median survival 7.5, 5.5, and 12.5 days, respectively; n = 6 for each group). However, in the LEW-to-ACI combination, animals with DST developed severe graft damage soon after transplantation and two of six (33.3%) animals died within 3 days, suggesting that antibody-mediated rejection occurred in these animals. Surprisingly, the same changes were observed when ACI animals transfused with third-party (PVG) blood received LEW intestinal grafts and three of six (50.0%) died within 3 days. Similar results were obtained after PVG-to-ACI transplants. Six of eight (75.0%) recipients with DST and one of six (16.7%) recipients with third-party (LEW) blood transfusion died within 3 days. Macroscopically, early graft damage was observed in all animals; however, some animals that were able to recover from this early damage showed prolonged survival.

LAb assay showed that sera from PVG blood transfused ACI rats were able to kill lymphocytes from donor (PVG) and all third-party strains (LEW, BN, and SD). When sera were absorbed with donor (PVG) RBCs to remove antibodies against donor MHC class I, LAb titer was slightly reduced from 2^{11} to 2^{10} against donor (PVG) and from 2^{10} to 2^9 against third-party (LEW) lymphocytes. The same degree of decrease in LAb titer was also seen after syngeneic RBC absorption, suggesting that the reduction after RBC absorption was not significant and was caused by sample dilution during the procedure.

All FK506-treated BN recipients of LEW grafts died of GVHD with a median survival of 32.0 days (n = 8). In contrast, when BN recipients received donor (LEW) and third-party (PVG) blood transfusion 7 days before grafting, animals did not show any signs of GVHD and survived for a median of 85.5 days (n = 12) and 92.0 days (n = 9), respectively. Four of 12 (33.3%) recipients pretreated with LEW blood transfusion and two of nine (22.2%) with PVG blood died of leakage or obstruction in intestinal anastomoses early after transplantation. These complications were thought to be an immunological event mediated by preformed antibodies. According to flow cytometric analysis, circulating donor lymphocytes reached 10% in untransfused recipients 6 to 7 days after SBTx, but donor or third-party blood transfusion totally eliminated donor lymphocytes from the circulation. These findings explained the absence of GVHD in blood-transfused animals.

From the Pittsburgh Transplantation Institute, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania.

Supported by research grants from the Veterans Administration and project grant No. DK29961 from the National Institutes of Health, Bethesda, Maryland.

Address reprint requests to Thomas E. Starzl, MD, PhD, Pittsburgh Transplantation Institute, University of Pittsburgh Medical Center, 3601 Fifth Ave, Pittsburgh, PA 15213.

0041-1345/94/$3.00/+0

DISCUSSION

Although LAb production after blood transfusion has been described,[3,4] damage caused by these antibodies was not reported after heart, kidney, or liver transplantation.[3,5,6] This study shows that hyperacute rejection of small bowel grafts occurs not only in donor-specific blood-transfused recipients, as reported by Gundlach et al,[5] but also in third-party blood-transfused recipients. Results of in vitro LAb assay confirm that antibodies produced in blood-transfused animals are not blood donor MHC class I specific and are cytotoxic to lymphocytes from many rat strains. LAbs induced by blood transfusion have been shown to be of both IgG and IgM class and have low affinity to kidney and liver tissues.[3,4] However, this study suggests that they may have high affinity to the lymphoid-rich small bowel graft.

A distinctive feature of small bowel grafts is the existence of specialized endothelial cells lining the high endothelial venules (HEV) in lymph nodes and Peyer's patches. Specific cell-to-cell recognition systems between lymphocytes and endothelial cells of HEV mediate migration of lymphocytes from the bloodstream to lymphoid tissues. LAbs produced by blood transfusion may have direct reactivity to endothelial cells of HEV.

The nature of antibodies induced by blood transfusion and the mechanisms of damage seen only in small bowel graft are under investigation.

REFERENCES

1. Murase N, Demetris AJ, Kim DG, et al: Surgery 108:880, 1990

2. Murase N, Demetris AJ, Woo J, et al: Transplantation 55:1, 1993

3. Furuya T, Murase N, Nakamura K, et al: Hepatology 16:1415, 1992

4. Downey WE III, Baldwin WM III, Sanfilippo F: Transplantation 49:160, 1990

5. Gundlach M, Oluwole SF, D'Agati V, et al: Transplantation 53:613, 1992

6. Hutchinson IV, Morris PJ: Transplantation 41:166, 1986