Simplified Rat Model of Intestinal Transplantation

Rat intestinal transplantation is an optimal model for research; nevertheless, it requires a complex and difficult microsurgical technique (*1–5*). We have recently developed a simplified rat model for intestinal transplantation using portal cuff anastomosis to overcome these technical difficulties (Fig. 1A).

Preoperative procedures, anesthesia and laparotomy are performed as previously described (*1–4*). In the donor operation, middle and descending colons are isolated from small bowel and ascending colon. Superior mesenteric vein is dissected and encircled. Splenic and pyloric veins are divided and portal vein is dissected up to hepatic hilum. Abdominal aorta is dissected and celiac trunk, right renal artery and lumbar branches are divided. Aorta is clamped at the diaphragm, tied below the mesenteric artery and this arterial segment is resected allowing a long aortomesenteric conduit. Entire small bowel, cecum and ascending colon are harvested on a vascular pedicle consisting of right colonic vessels, aortomesenteric conduit and portal vein. The graft is flushed and stored in cold preservation solution. Polyethylene catheter is used to create the cuff as described elsewhere (*5*). In this modified technique, the vein is introduced into the cuff and everted, covering the outer wall and fixed with three equidistant stitches (Fig. 1B).

In the recipient, the superior mesenteric vein is exposed, and a segment between the first and second jejunal branch is dissected and prepared for cuff anastomosis. Eventually, the second jejunal branches and the right colonic vein may be divided in order to increase this segment. The graft is implanted in the recipient with an end-to-side aorto-aorta anastomosis as previously described (*1–5*). The dissected segment of mesenteric vein is isolated between clamps to insert the portal cuff and a 6-0 suture is used to fix the cuff into recipient's mesenteric vein. The clamps are released and immediate graft reperfusion is observed. The recipient jejunum, ileum, cecum, and ascending colon are removed en bloc. Finally, anastomosis between the graft and the remaining intestine restores the digestive tract (Fig. 1A).

The present model offers many possibilities for intestinal transplantation research with the following advantages: reconstitutes the natural and physiologic portal graft drainage; the portal cuff anastomosis is performed within few minutes without bleeding or venous thrombosis. This alternative technique avoids recipient visceral ischemia and hemodynamic instability because there is no obstruction on inferior vena cava and portal vein. Furthermore, it maintains grafts ileocecal valve and preserves the extrinsic enervation along the mesenteric-aortic segment that may improve bowel movement.

This modified technique can be easily performed within two hours after a short period of training (8±3 week) and may reach excellent success rates. This rat model may surmount technical obstacles precluding microsurgical techniques and may ultimately spread this model in centers with low technical resources.

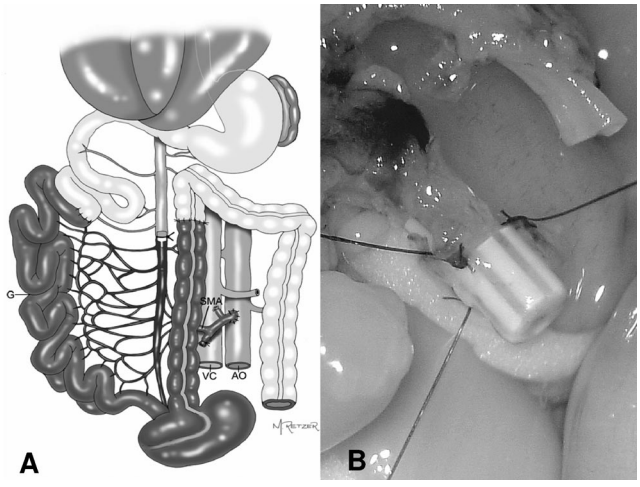

FIGURE 1. (A) Figure illustrating the present model of intestinal transplantation. G, intestinal graft in shady color; SMA, superior mesenteric artery of the graft; VC, recipient vena cava; AO, recipient aorta. (B) Micropicture (16×) showing details of the portal cuff.

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This study was supported by Sao Paulo State Council of Research (FAPESP): 98/09714-4.
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Received 26 January 2005.
Accepted 13 April 2005.

ISSN 0041-1337/05/8010-1522
DOI: 10.1097/01.tp.0000184448.86704.c8

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γδ T Cells and Resolution of Cytomegalovirus Infection in an HIV/HCV Coinfected Patient after Liver Transplantation

Cytomegalovirus (CMV) infection is a frequent transplant complication. Anti-CMV responses in allograft recipients have been extensively studied, only few reports addressing γδ T lymphocytes role (*1*, *2*). γδ T cells may play a natural antiviral role (*3*), and kidney allograft recipients during CMV infection undergo a massive expansion of circulating γδ T cells (*4*). The occurrence of a primary CMV infection in a highly immunosuppressed patient may represent a model about the role of innate immunity in this setting.

We analyzed a 41-year-old human immunodeficiency virus (HIV)/hepatitis C virus (HCV) coinfected patient undergoing primary CMV infection after orthotopic liver transplantation (OLT). The donor was negative for antibodies to HIV-1/2 or HCV, but positive for CMV IgG. At day 42 after OLT, the patient suffered from fever (>38°C for more than 2 days), leukopenia ($<4\times10^9$ leukocytes/L in >2 consecutive samples) and malaise. CMV infection resolved within day 60. No CMV prophylaxis or treatment were performed and CMV infection was retrospectively diagnosed. Thus, resolution was only dependent on the host-response. At day 45, CMV-DNA was transiently positive; anti-CMV IgM and IgG were observed at days 99 and 112, respectively.

As Vδ1 T lymphocytes are specifically expanded by both chronic HIV (*5*, *6*) or acute CMV infections (*1*, *2*), the distribution of this γδ T cell subset was compared to the kinetics of CMV- and HIV-specific CD8 T cell responses (Fig. 1). Lymphocytes showed a transient decrease at CMV viremia and disease onset (day 45, Fig. 1A). Vδ1 T cells increased at day 2, decreased at the time of clinical disease at day 45 and dropped at day 72, suggesting a γδ-specific T cell exhaustion and/or tissue migration. A subsequent increase of Vδ1 T cells was ob-

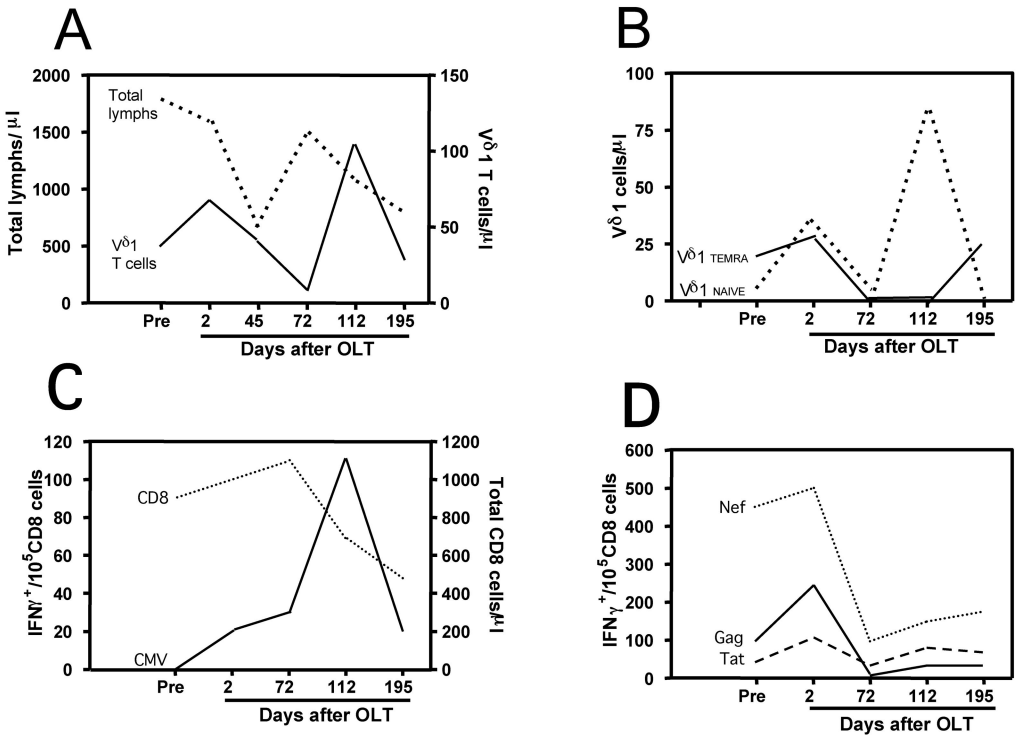

FIGURE 1. Vδ1 T lymphocytes and CMV- and HIV-specific CD8 responses in an HIV/HCV coinfected patient undergoing acute CMV infection after OLT. (A) Circulating total lymphocyte number and Vδ1 T cells number are shown. (B) Vδ1 differentiation subsets are shown as determined by flow cytometry. In particular, Vδ1 Naïve CD27+CD45RA+ and terminally differentiated effector memory CD27-CD45RA+ cells (Temra) numbers are shown. (C) CMV-specific CD8 response to CMV antigens, measured as IFN-γ production, is shown. (D) HIV-specific CD8 response to Nef, Gag, and Tat HIV-1 proteins, measured as IFN-γ production by flow cytometry, are shown. In both panels C and D, IFN-γ producing cell numbers among 10^5 CD8 T cells are shown.