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Chronic Rejection after Rat Liver–Small Bowel Transplantation

Detlef Meyer, Christoph Otto, Martin Gasser, Uwe Heemann, Karin Ulrichs, and Arnulf Thiede

The pathogenesis of chronic rejection after small bowel transplantation is still not well understood. Statistically, however, chronic allograft dysfunction in humans seems less frequent if small bowel is transplanted in combination with a liver. Therefore, the aim of this study is to establish an experimental model for investigation of this phenomenon. Results after combined liver–small bowel transplantation (with or without immunosuppression) have been compared with isolated small bowel transplantation in the Brown Norway-Lewis (BN-LEW) rat strain combination. Signs of rejection were evaluated by histology, immunohistochemistry, and the rate of parenchymal apoptosis in the allografts. Graft survival after liver–small bowel transplantation was prolonged in concomitant liver–small bowel transplantation as compared with small bowel transplantation alone. Furthermore, not only less immunosuppression was needed to establish long-term acceptance after a transient acute rejection, but tolerance was established after liver–small bowel transplantation. However, without initial immunosuppression, a more severe acute rejection crisis resulted in chronic rejection even after liver–small bowel transplantation. Concordant to results after human renal transplantation, the degree of the early parenchymal injury in the allografts seems to be a reliable predictor for the late onset of chronic rejection.

Introduction

Several follow-up studies after human renal transplantation demonstrate that early acute rejection episodes accelerate the onset of chronic rejection in the late postoperative period. Although acute rejection can be sufficiently treated by standard immunosuppressive protocols, it does not influence the onset of chronic rejection. In fact, this seems to emphasize the hypothesis of two different mechanisms that independently develop after organ transplantation. However, the onset of chronic small bowel rejection in humans is positively modulated by a concomitant liver transplantation. The aim of our study therefore was to establish an experimental model that reflects clinical conditions and evaluates the relation between acute and chronic rejection after small bowel transplantation.

Materials and Methods

In a fully major histocompatibility complex-incompatible Brown Norway (BN) (RT1)–Lewis (LEW) (RT1) rat strain combination, 2 experimental models were used to compare isolated small bowel transplantation with combined liver–small bowel transplantation (Table 1). Indicator heart and skin transplantations were performed to investigate the development of donor-specific tolerance in long-term surviving recipients. Sequential histology, immunohistochemistry (Table 2), and a TUNEL-assay were used to further analyze the cellular changes in the allografts. The project was approved by the ethics committee to fulfill the German law on the protection of animals.

Results

Orthotopic Small Bowel Transplantation (OSBTx) versus Liver–Orthotopic Small Bowel Transplantation (LOSBTx)

Initial high-dose immunosuppression provided a sufficient recovery after OSBTx in the early postoperative period. However, all recipients devel-
OPED DIARRHEA IN THE 12TH POSTOPERATIVE WEEK AS A SIGN OF ALLOGRAFT DYSFUNCTION AND DIED AROUND POSTOPERATIVE DAY (POD) +100 (FIG 1). HISTOLOGY REVEALED THE TYPICAL PATCHY APPEARANCE OF MUCOSAL ULCERS AND SUBMUCOSAL TRANSPLANT VASCULOPATHY, WHICH INDICATE CHRONIC ALLOGRAFT DYSFUNCTION (FIG 2). IN CONTRAST, RECIPIENTS OF A COMBINED ALLOGRAFT (LOSBTx) SURVIVED INDEFINITELY (> 150 DAYS) IN 80% OF THE CASES (FIG 1), ALTHOUGH THE INITIAL IMMUNOSUPPRESSIVE DOSAGE WAS DECREASED TO 25%. THEIR CLINICAL COURSE WAS COMPLETELY UNEVENTFUL. HOWEVER, SEQUENTIAL HISTOLOGY AND IMMUNOHISTOCHEMISTRY DEMONSTRATED SIGNS OF ACUTE REJECTION IN THE 4TH POSTOPERATIVE WEEK, WHICH RESULTED IN AN INCREASE OF APOPTOTIC PARENCHYMAL CELLS AT THAT TIME. AT POD +150, THE NUMBER OF INFILTRATING CELLS AND THE RATE OF APOPTOSIS WAS DECREASED TO NORMAL LIMITS (FIG 3). FURTHERMORE, INDEFINITE SURVIVAL OF DONOR-RESTRICTED HEART AND SKIN TRANSPLANTS SECONDARILY TRANSPLANTED AT POD +100 AFTER LOSBTxS INDICATED TOLERANCE INDUCTION (DATA NOT SHOWN).

**Table 1 | Experimental Groups and Their Immunosuppressive Therapy**

<table>
<thead>
<tr>
<th>Strain Combination</th>
<th>Transplantation</th>
<th>Immunosuppression</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>BN-LEW</td>
<td>OSBTx</td>
<td>2.0 mg FK506/kg/d; days 0–5</td>
<td>29</td>
</tr>
<tr>
<td>BN-LEW</td>
<td>LOSBTx</td>
<td>0.5 mg FK506/kg/d; days 0–5</td>
<td>34</td>
</tr>
<tr>
<td>BN-LEW</td>
<td>HSBTx</td>
<td>no FK506</td>
<td>9</td>
</tr>
<tr>
<td>BN-LEW</td>
<td>LHSBTx</td>
<td>no FK506</td>
<td>32</td>
</tr>
</tbody>
</table>

**Table 2 | Monoclonal Antibodies (mAb, Serotec, Oxford, UK) Used for Immunohistochemistry**

<table>
<thead>
<tr>
<th>mAb</th>
<th>Cluster</th>
<th>Cell Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ox42</td>
<td>CD11 b+c</td>
<td>Macrophages</td>
</tr>
<tr>
<td>W3/25</td>
<td>CD4</td>
<td>CD4+ T lymphocytes, macrophages</td>
</tr>
<tr>
<td>Ox8</td>
<td>CD8, α-Kette</td>
<td>CD8+ T lymphocytes, natural killer cells</td>
</tr>
<tr>
<td>Ox39</td>
<td>CD25</td>
<td>Interleukin-2-receptor of activated T lymphocytes</td>
</tr>
<tr>
<td>NOS61</td>
<td>CD25</td>
<td>Interleukin-2-receptor of activated T lymphocytes</td>
</tr>
</tbody>
</table>

**Heterotopic Small Bowel Transplantation (HSBTx) versus Liver–Heterotopic Small Bowel Transplantation (LHSBTx)**

As expected, all recipients after HSBTxs without immunosuppression died within 3 weeks due to complications in a severe acute rejection crisis confirmed by histology (Fig 1). Transplanting a liver together with the small bowel (LHSBTx) increased allograft acceptance in the BN recipients: 70% (14/20) of these recipients survived long-term without any immunosuppressive treatment (Fig 1). In contrast to the results after LOSBTxs, the clinical course of severe weight loss during the first 4 postoperative weeks indicated an acute rejection crisis, which was confirmed by histology. The intense cellular infiltration consisted of macrophages and activated CD4+ and CD8+ T lymphocytes (Fig 4). Parenchymal cell damage increased significantly during that period. But until POD +100, histological signs of rejection resolved spontaneously. Goblet cells reoccurred in the small bowel epithelium as a sign for the regained functional integrity of the allografts. How-
Figure 1. Survival rate after heterotopic small bowel transplantation (HSBTx), orthotopic liver–heterotopic small bowel transplantation (LHSBTx), orthotopic small bowel transplantation (OSBTx), orthotopic liver–small bowel transplantation (LOSBTx).

Figure 2. Intimal thickening (→) of the submucosal vessels of the small bowel allograft at postoperative day +90 after orthotopic small bowel transplantation (OSBTx); H&E histology, ×400.
Figure 3. Liver allograft after orthotopic liver–small bowel transplantation (LOSBTx), (a) mononuclear infiltration in the portal triad at postoperative day (POD) +14; (b) portal triad at POD +150. Small bowel allograft after LOSBTx: (c) mononuclear infiltration in the crypts at POD +14; (d) intact intestinal epithelium at POD +150; H&E histology, × 200.

Figure 4. CD8+ T lymphocytes in the portal triad of the liver allograft after orthotopic liver–small bowel transplantation (LOSBTx) at (a) postoperative day (POD) +14 and (b) POD +150; in the small bowel allograft after LOSBTx; (c) at POD +14; and (d) at POD +150; immunohistochemistry, × 200.
ever, 10% (2/14) of the long-term surviving recipients developed histological signs of chronic rejection located in the portal triad of the liver (Fig 5) and in the submucosa of the small bowel allografts (Fig 6). Tissue fibrosis and transplant vasculopathy (intimal proliferation and thickening of the muscular layer with a narrowing of the vascular lumen by 40%–60%; data not shown) were found.

Discussion
Comparing the results after OSBTx and LOSBTx, our data indicate that a concomitant liver transplantation increases small bowel allograft acceptance in this particular experimental setting. The liver significantly reduces the immunosuppressive dosage, which is needed for successful outcome after small bowel transplantation and prevents acute rejection. However, the liver allograft itself does not directly inhibit chronic rejection. After LOSBTx (without immunosuppression), chronic rejection of the orthotopically placed small bowel is prevented. However, after LHSBTx (without immunosuppression), chronic rejection of the heterotopically placed small bowel occurred in 10% of the long-term surviving recipients. In his studies on rat small bowel transplantation, Su described that histological and electrophysiological signs of chronic rejection were more likely to occur in allografts that have been placed heterotopically.7 The lacking nutritional transit in this model may influence the immune response of the recipient. However, histological signs of chronic rejection also occurred after orthotopic small bowel transplantation in this study. From our experience, the most reliable factor to predict chronic rejection appears to be the tissue damage in the allografts caused by acute rejection episodes in the early postoperative period. At a certain degree of parenchymal destruction, the allograft loses its potency for a “restitutio ad integrum.” Tissue damage resulting from the reperfusion in-

**Figure 5.** Histological signs of chronic rejection in the liver allograft at postoperative day +100 after orthotopic liver–heterotopic small bowel transplantation (LHSBTx): (a) intimal proliferation in a vessel of the portal triad (→); H&E histology, × 400; (b) fibrosis and bile duct proliferation of the portal triad (→); H&E histology, × 100.

**PARENCHYMAL APOPTOSIS**
Programmed cell death actively induced by a stimulus (eg, Fas Ligand) that results in DNA fragmentation of the nucleus in the parenchymal cells of the allograft.
Donor-specific tolerance

Immunological non-reactivity to the allograft with preservation of the intact immune system.

1. We demonstrated that concomitant liver transplantation facilitates small bowel allograft acceptance (LOSBTx), decreases the immunosuppressive dosage needed for the successful outcome after small bowel transplantation, and induces tolerance.

2. We established a model for chronic rejection after liver–small bowel transplantation (LHSBTx).

3. We confirmed our hypothesis that the onset of chronic rejection is apparently related to the tissue damage caused by an acute rejection crisis during the early postoperative period.

Further studies are needed to investigate the mechanisms linking these 2 rejection processes.

References


Figure 6. Histological signs of chronic rejection in the small bowel allograft at postoperative day +100 after orthotopic liver-heterotopic small bowel transplantation (LHSBTx): (a) intimal proliferation in a submucosal vessel (→); H&E histology, × 400; (b) villous blunting and thickening of the muscular layer; H&E histology, × 100.