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"Tolerogenic effect" of the liver for a small bowel allograft

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Abstract A newly developed liver/small bowel transplantation model (LSBTx) was used to investigate the tolerogenic effect of a liver allograft toward a simultaneously transplanted small bowel. Small bowel transplantation (SBTx) under high-dose immunosuppression was compared to LSBTx with a lower FK506 dosage. Syngeneic Lewis [(LEW) to LEW] and two fully allogeneic rat strain combinations (Brown Norway-to-LEW and Dark Agouti-to-LEW) were used. Clinical course and histological findings after SBTx demonstrated a chronic rejection of the small bowel allograft within 100 days. However, after LSBTx long-term acceptance (> 150 days) was achieved after a transient rejection crisis, although initial immunosuppression was significantly lower. Furthermore, indicator heart transplantations demonstrated the induction of donor-specific tolerance in both allogeneic strain combinations. In contrast to other LSBTx rat models, these results reflect observations after human LSBTx, in which the rate of acute and chronic rejection is also significantly lower than after human SBTx.

Key words Liver transplantation · Small bowel transplantation · Tolerogenicity

Introduction

The beneficial effect of a liver allograft toward another simultaneously transplanted organ is well known since first described by Calne in a pig model for combined liver/kidney transplantation [2]. Graft survival after human kidney transplantation is also significantly prolonged by a concomitant liver transplantation [12]. Furthermore, acute rejection of a pancreas allograft is reversed by a secondarily transplanted liver in a rat model [15]. Recent data for human liver/small bowel transplantation (LSBTx) also indicate a beneficial effect of the concomitant liver allograft: the rate of severe acute rejection as well as chronic rejection after small bowel transplantation (SBTx) is significantly reduced if the small bowel is transplanted together with a liver allograft [6, 13]. However, experimental data did not reflect these clinical findings so far. Small bowel alone or transplanted together with a liver allograft were invariably rejected in rats [17]. Survival rates in a pig model combining liver with small/large bowel transplantation were even more disappointing: only 11 % of the recipients survived after combined transplantation compared to 100 % after isolated small bowel transplantation [5].

In order to investigate the clinically suspected beneficial effect of a liver toward a small bowel allograft, we developed a new rat model for combined LSBTx. The orthotopically placed, arterialized liver is combined with a small bowel graft of the same donor, which is also orthotopically placed and drained via the portal vein into the liver graft. Physiological blood supply of the combined graft and the fact that both transplants, liver and small bowel, originate from the same donor are the major advantages of this new model.

Table 1 Immunosuppression and survival rates after small bowel transplantation *(SBTx)* and liver/small bowel transplantation *(LSBTx)* in syn- and allogeneic strain combinations. (*LEW* Lewis, *BN* Brown Norway, *DA* Dark Agouti)

Transplantation	Group	Strain combination	*n*	Immunosuppression (FK506)	Survival (days)
SBTx	1	LEW–LEW	5	None	> 150 (5×)
SBTx	2	BN–LEW	14	2.0 mg/kg per day, days 0–5	95, 96, 96, 98, 102
SBTx	3	DA–LEW	14	2.0 mg/kg per day, days 0–9	90, 91, 96, 97, 99
LSBTx	4	LEW–LEW	5	None	> 150 (5×)
LSBTx	5	BN–LEW	25	0.5 mg/kg per day, days 0–5	28, 102, > 150 (8×)
LSBTx	6	DA–LEW	22	1.0 mg/kg per day, days 0–9	12, 71, 104, > 150 (4×)

Material and methods

Results after isolated SBTx were compared to the findings after combined LSBTx in two fully allogeneic rat strain combinations: the intermediate-responder combination Brown Norway (BN, $RT1^n$-to-Lewis (LEW, $RT1^l$) and the high-responder combination Dark Agouti (DA, $RT1^{avl}$)-to-LEW. Allograft recipients were treated with different dosages of FK506 (Table 1). Syngeneic recipients without immunosuppression served as controls. Sequential histology (hematoxylin and eosin) and immunohistochemistry served to investigate pathological changes in the graft. The following antibodies were used: NDS60 (MHC class I, LEW), W3/25 (CD4 + T lymphocytes), Ox8 (CD8 + T lymphocytes), and Ox42 (macrophages) (Serotec, England). On day + 70 after LSBTx donor- and third-party hearts were transplanted heterotopically into the abdomen to verify induction of donor-specific tolerance.

Results

Survival rate

After excluding technical failures (death of the recipients until day + 5), 100% long-term survival (> 150 days) was achieved in both models, after syngeneic SBTx and LSBTx. In the intermediate responder combination BN-to-LEW treated with high-dose immunosuppression (group 2) isolated small bowel allografts functioned for the first 10 postoperative weeks. However, beyond day + 80 all recipients developed diarrhea with a fatal outcome until day + 102 at the latest (median survival: 97.4 days). In contrast, 80% long-term survival was achieved in the same strain combination after LSBTx with a four times lower FK506 dosage (group 5). Similar results were observed in the high responder combination DA-to-LEW: all recipients of an isolated small bowel graft (group 3) developed diarrhea in the late postoperative period and died until day + 99 at the latest (median survival: 94.6 days) despite an initially high-dose immunosuppression. Combining small bowel and liver transplantation (group 6), half of the FK506 dosage was sufficient to achieve long-term acceptance in 58% of the cases (Table 1).

Indicator heart transplantation

Long-term survivors after allogeneic LSBTx (groups 5 and 6) received a heterotopic cardiac allograft on day + 70 ($n = 6$ each group). In group 5, donor-specific BN hearts were accepted long term (> 100 days), whereas third-party cardiac allografts (DA) were rejected within 7 days. In group 6, similar observations were made: donor-identical hearts (DA) functioned indefinitely, whereas third-party hearts (BN) were regularly rejected.

Histology

After allogeneic SBTx, a permanent mononuclear cell infiltration was observed in the crypts and along the lamina propria of the small bowel allografts beyond day + 28. In the late postoperative period, fibrosis of microvilli was generally seen. Infrequently perivascular fibrosis and hyperplasia of the intima were observed, especially in arteries of the submucosa and the mesentery. In contrast, histology on day + 28 after LSBTx showed only a minor infiltration of mononuclear cells in crypts of the small bowel allografts and in the portal area of the liver allografts. This infiltration is resolved until day + 150. The small bowel epithelium in particular contained the normal amount of goblet cells. No blebbing of the tips of microvilli, a typical sign of rejection, was observed. No differences in graft histology between the two allogeneic strain combinations (groups 2 and 3, and groups 5 and 6) could be found.

Immunohistochemistry

The mononuclear cell infiltrate in small bowel allografts (groups 2 and 3) after day + 28 contained mostly CD4 + and CD8 + T lymphocytes and macrophages of recipient origin. After LSBTx, the same leukocyte subpopulations were identified, but CD8 + T lymphocytes disappeared until day + 150 in both allogeneic strain combinations.

Discussion

Summarizing the results, small bowel allografts develop histological signs of chronic rejection (fibrosis of microvilli, vasculopathy) in the late postoperative period. Graft dysfunction appears clinically as diarrhea and results in death of the recipients. These pathological changes occur despite initial high-dose immunosuppression. By combining liver and small bowel transplantation, chronic rejection can be prevented in 80% of the cases in the intermediate-responder group (group 5) and in almost 60% of the cases in the high-responder combination (group 6). Furthermore, donor-specific tolerance is induced. This can be achieved with a significantly reduced immunosuppression in both fully allogeneic strain combinations.

Different models for liver and small bowel transplantation are described in the literature. Zhong used a non-arterialized liver allograft [17]. The small bowel graft was obtained from a second but genetically identical donor and drained via the vena cava of the recipient. Acute rejection of both allografts occurred after 10 days. These results, therefore, lend no support to the hypothesis of a beneficial effect of concomitant liver transplantation. Kobayashi and Sarnacki transplanted a small bowel after donor-identical liver allograft had already established tolerance in the recipients immune system [8, 14]. Long-term acceptance was thus achieved. This sequential transplantation model illustrates the capacity of the liver to induce tolerance in the rat, but implications for human combined LSBTx are limited. To our knowledge, the demonstrated model (LSBTx) documents for the first time the tolerogenic effect of a rat liver allograft towards a *simultaneously* transplanted small bowel.

The physiological blood supply of a combined allograft in this particular model seems to be important for the observed tolerogenic effect of the liver. The first liver transplantation model in rats was developed by Lee and modified by Kamada to include non-arterialized liver allografts [7, 9]. Engemann demonstrated that these non-physiologically perfused liver allografts show a chronic inflammatory reaction as well as frequent ischemic changes of the bile ducts [3]. These pathological changes do not appear if the donor hepatic artery is reconstructed during transplantation. Furthermore, spontaneous tolerance develops in 80% of the cases in the BN-to-LEW strain combination. Similar findings can be observed after LSBTx. Long-term acceptance and donor-specific tolerance do not occur after combined SBTx with a non-arterialized liver allograft [17]. However, donor-specific tolerance is achieved in our model using an arterialized liver allograft (shown by indicator heart transplantation). Therefore, we conclude that tolerogenicity of the liver allograft seems to depend on physiologically perfused liver tissue.

Which compartment or cell type of the liver induces tolerance is not identified yet. Although different theories have been investigated, each one of them alone does not explain completely the unique effect of a liver allograft on the recipients immune system:

1. Hepatocytes secrete an increasing amount of soluble MHC-I molecules during transplantation, but soluble MHC-I molecules alone do not induce tolerance [16].
2. Donor-derived stem cells are transplanted together with the liver in considerable number compared to other organ transplantations (for example, heart, kidney), but dendritic cells, which originate from these stem cells, fail to induce tolerance in vitro in contrast to bone marrow-derived dendritic cells [10].
3. Apoptosis of alloreactive T lymphocytes can be induced by rat Sertoli cells of the testis [1] and rat epithelial cells of the anterior chamber of the eye [4]. This avoids rejection of these tissues in an experimental setting. Similar findings are observed in liver allografts during tolerance induction [11]. But the underlying mechanism in the liver is so far not identified.

However, different factors in the liver are apparently involved in its tolerogenic capacity. A combination of these factors plus a physiological perfusion of the liver graft seems to change the initial immune response of the recipient from rejection to tolerance. This occurs whether the liver is transplanted alone or in combination with other organs.

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