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Donor-derived alloantigen-presenting cells persist in the liver allograft during tolerance induction

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Abstract The predictive value of chimerism was evaluated in three different transplantation models in the rat without immunosuppression: small bowel- (SBTx), liver- (LTx), and liver/small bowel transplantation (LSBTx) were performed in the Brown Norway (BN)-to-Lewis-(LEW) strain combination. Immunohistochemistry and flow cytometry were used to identify donor cells in the recipient's spleen. Their number did not change significantly during transient rejection or tolerance after LTx and LSBTx. However, the amount of donor-derived nonparenchymal cells within the liver allograft including antigen-presenting cells (APCs), such as dendritic and Kupffer cells, clearly mirrored the recipient's immune status: as expected, their number decreased during rejection, but recovered considerably during and after tolerance induction. We conclude that donor cells in the periphery of the recipient correlate with the presence of the allograft, but do not seem to influence graft acceptance actively. However, the kinetics of the detected donor APC population in the liver suggests their important role in modifying the recipient's immune response towards tolerance.

Key words Liver/small bowel transplantation · Tolerance · Dendritic cells · Kupffer cells · Antigen-presenting cells · Chimerism

Introduction

The phenomenon of chimerism is well-known since male donor cells, identified after cadaveric liver transplantation by their Y-chromosome, were observed in female recipients [14]. Much attention was paid to this phenomenon after the extent of chimerism was related to the duration of allograft survival [36]. Experimental studies demonstrated the need for a certain amount of donor cells in the periphery of the recipient to provide better graft acceptance [15]. Based on these considerations, clinical studies were performed, combining solid organ grafting with bone marrow transplantation [27]. However, recent reports on allograft rejection despite successfully established chimerism [22, 30] demonstrated the need for further research on this phenomenon.

Chimerism, with its simultaneous detection of donor and recipient cells, appeared to follow a distinct pattern. So-called passenger leukocytes, transferred into the recipient together with the graft, could be detected in the recipient's blood for approximately 3 weeks after human liver transplantation (*macrochimerism*) [29]. These cells invariably disappeared from the blood stream, presumably due to an active immune response of the recipient. At a later stage, donor cells, most likely produced by stem cells cotransplanted with the graft, were detected in peripheral organs (e.g., spleen and skin) of long-term survivors (*microchimerism*). Irrespective of the point of time after transplantation, the coexistence of donor and recipient leukocytes within the allograft is described as *graft chimerism*. However, since a complete exchange of donor leukocytes was thought to occur in

the allograft during rejection [31], graft chimerism appeared to be a short-term phenomenon.

To investigate the relationship between allograft survival and these three different types of chimerism, transplantation models in the rat were selected, where the immune response was not influenced by an immunosuppressive therapy: in the Brown Norway (BN)-to-Lewis (LEW) strain combination, small bowel transplantation (SBTx) leads to lethal rejection of the allograft; in contrast, after liver transplantation (LTx) using the same strain combination, tolerance towards the liver is achieved, while combined liver/small bowel transplantation (LSBTx) results in tolerance towards the combined allograft [21]. The "tolerogenic capacity" of the liver has also been investigated in combination with other organs, e.g. the pancreas or kidney [3, 43]. The small bowel was chosen in this particular study for two reasons:

(1) It provokes a strong rejection response, which causes severe complications in human SBTx [10]. Experimental solutions for this problem would therefore have direct clinical implications.
(2) The small bowel releases a high number of passenger leukocytes into the recipient and should consequently induce a high level of chimerism.

Therefore, the value of chimerism for rejection or tolerance under these experimental conditions will be of special interest.

Materials and methods

Experimental approach

LTx ($n = 25$) was performed in an orthotopic, arterialized model as published earlier [41]. For SBTx ($n = 16$) a heterotopic model with a portal venous drainage was used. LSBTx ($n = 25$) was performed in an en-bloc procedure using an arterialized liver and a heterotopic small bowel allograft draining via the portal vein. These allogeneic transplantations were performed in the BN-to-LEW strain combination. None of the graft recipients received immunosuppressive therapy. LEW-to-LEW transplantations ($n = 17$ in the LTx and LSBTx groups, $n = 11$ in the SBTx group) served as syngeneic controls. Death of animals before day + 4 was attributed to technical errors and led to exclusion from this study. Body weight and extent of jaundice were screened daily. Animals were sacrificed on days + 3, + 7, + 14, + 28, and + 100 in all groups except for the SBTx group, in which all animals died during the first 3 weeks. Organ harvest in this group was performed not later than day + 7 since lethal rejection completely destroyed small bowel allografts. To test for immunological tolerance, BN donor hearts were transplanted heterotopically into the recipient's abdomen at day + 70 following LTx and LSBTx. DA hearts served as third-party controls. The project was approved by the ethic committee to comply with the German law on the protection of animals.

Flow cytometric analysis

Blood and tissue sections of the spleen and mesenteric lymph nodes were harvested for flow-cytometric analyses. An erythrocyte-free leukocyte suspension was collected after short incubation with 0.9% hypotonic ammonium chloride solution, and washed twice with phosphate-buffered saline (PBS, pH 7.4). The specimens of spleen and lymph nodes were mashed through a fine steel sieve and filtered through a nylon mesh (100 µm pore size) to remove organ fragments. Cell suspensions of spleen, lymph nodes, and hemolyzed blood were characterized using the following mouse monoclonal antibodies (mAbs) (Serotec, Oxford, UK): R73 (α/β T cells), O×8 ($CD8^+$ T cells, α-chain), 341 ($CD8^+$ T cells, β-chain), W3/25 ($CD4^+$ T cells), Ox33 (B cells), ED2 (tissue macrophages, Kupffer cells), O×42 (macrophages), O×62 (dendritic cells), O×27 ($RT1^n$, BN rat strain), NDS60 ($RT1^l$, LEW rat strain; kindly provided by M. Dallman, London, UK). All mAbs were used in saturated concentrations.

For two-color-fluorescence analysis, 5×10^5 cells were incubated with an appropriate dilution of strain-specific unlabeled mAbs (O×27, NDS60) in a total volume of 100 µl. All staining steps were performed at 4 °C for 20 min. After washing with PBS, the cells were incubated with rabbit-anti-mouse IgG, PE-conjugated $F(ab')_2$ (Serotec). Before adding cell subset-specific fluroreceinisothiocyanate-labeled mAbs (W3/25, O×8, O×33), a blocking step with mouse serum (1:10 dilution) was performed. After washing, cells were analyzed by flow cytometry. Using a FAC-scan flow cytometer (Becton Dickinson, Heidelberg, Germany), a total of 10,000 cells was counted for each analysis in the lymphocyte life gate defined by forward and side scatter profile. Dead cells and erythrocytes were excluded from acquisition. Data analysis was performed using LYSIS II software (Becton Dickinson). The instrument setting was identical for all experiments.

Histology, immunohistochemistry

Specimens of the spleen, mesenteric lymph nodes, liver, and small bowel grafts were fixed in buffered formalin, embedded in paraffin, and cut into 2 µm-sections for hematoxylin-eosin (H&E) staining. The evaluation of the H&E sections was performed semiquantatively. For immunohistochemistry, 4 µm-cryosections obtained from each organ were stained with mAbs O×27, NDS60, R73, 341, and O×62 using a three-layer immune peroxidase technique, and finally counterstained with hematoxylin. Endogenous peroxidase was quenched with 3% hydrogen peroxide for 1 h.

Double staining procedure

Monoclonal antibodies for leukocyte subsets were used in combination with the donor cell marker (mAb O×27). Previously-labeled mAb R73, O×62, or ED2 sections were further incubated with O×27 for 1 h at 37 °C and visualized by a streptavidin-biotin-system (SuperSensitiveKit, Biogenex, San Ramon, Calif. USA) labeled with alkaline phosphatase. Fast red was used as substrate, followed by counterstaining with hematoxylin. Positive cells were counted under a microscope in 10 randomly-chosen high-power fields (× 400 magnification).

Table 1 Survival after liver *(LTx)*, small bowel *(SBTx)*, and combined liver/small bowel transplantation *(LSBTx)* in the BN-to-LEW strain combination

Transplantation	Strain combination	*n*	Survival time (days)
LSBTx	BN-to-LEW	10	14, 17, 24, > 100 (7 ×)
LSBTx	LEW-to-LEW	5	> 100 (5 ×)
LTx	BN-to-LEW	10	24, 68, > 100 (8 ×)
LTx	LEW-to-LEW	5	> 100 (5 ×)
SBTx	BN-to-LEW	10	8, 8, 8, 8, 9, 10, 11, 14, 19, 20
SBTx	LEW-to-LEW	5	> 100 (5 ×)

Statistical analysis

Statistical significance was evaluated using the Student's *t* test. After normal distribution of the data was confirmed, significance was established at a *P* value of less than 0.05.

Results

Clinical outcome

Survival data after allo- and syngeneic transplantation are summarized in Table 1. Isolated LTx resulted in a long-term survival rate of 80%. Animals on which SBTx had been performed died within 3 weeks after transplantation. Interestingly, after combined LSBTx long-term survival was achieved in 70% of the animals. Syngeneic controls survived indefinitely without signs of graft injury. Following LTx, clinical signs of rejection, i.e., loss of body weight and jaundice (Fig. 1), were restricted to the first 2 postoperative weeks. Following this period, these animals recovered without any sign of further liver damage. Similarly, a limited period of weight loss and jaundice was observed after LSBTx. However, it appeared to be clinically more severe and resulted in a survival rate 10% lower than that in the case of LTx. H&E histology confirmed clinical observations of a self-limited rejection after LTx and LSBTx (Fig. 2).

To assess the development of immunological tolerance, heart transplantation was performed on day + 70 after LTx and LSBTx, respectively. Heterotopic BN hearts (donor type) were accepted indefinitely (> 100 days), while DA hearts (third party) were rejected in the normal range (Table 2). Thus, induction of immunological tolerance towards donor alloantigens was achieved in both transplantation models, LTx and LSBTx.

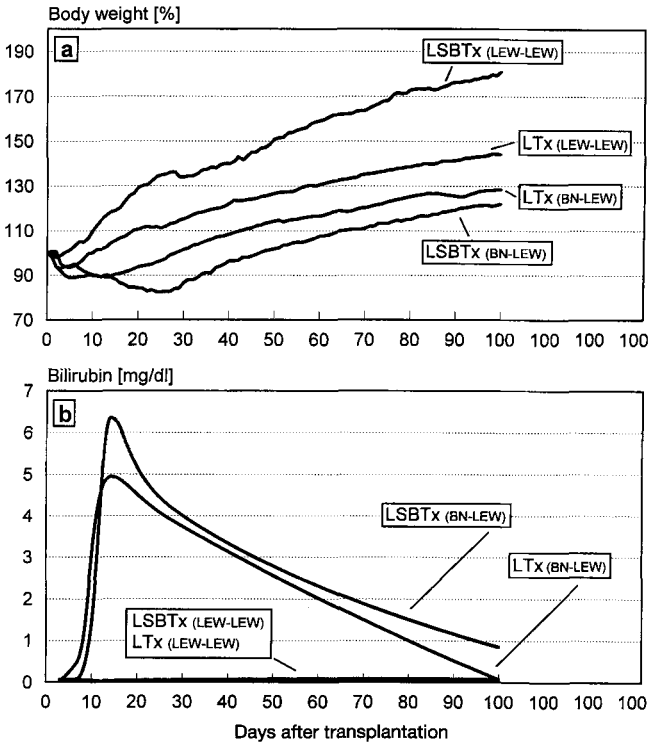

Fig. 1 Mean values of (**a**) body weight and (**b**) serum-bilirubin after syngeneic (LEW-to-LEW, $n = 5$ for each group) and allogeneic (BN-to-LEW, $n = 10$ for each group) liver (*LTx*) and combined liver/small bowel transplantation (*LSBTx*)

Macrochimerism

Macrochimerism in the allograft recipients was investigated during the early postoperative period (day + 3 and day + 7, respectively). Using two-color fluorescence analysis, donor $CD8^+$ T cells, B cells, and $CD4^+$ T cells were observed in the recipient's blood, spleen, and mesenteric lymph nodes (Table 3). Three days after both, LSBTx and SBTx, significantly higher numbers of donor cells than in the case of LTx were detected in all three compartments. Donor lymphocytes consisted predominantly of $CD4^+$ T cells, and to a lesser extent of B cells and $CD8^+$ T cells. At day + 7 however, donor lymphocytes decreased significantly in the LSBTx and SBTx groups, to a level comparable to that in the LTx groups. In contrast to the cell decrease in the LSBTx and SBTx groups, the number of donor cells in the LTx group remained almost unchanged. Obviously, in the early stage small bowel allografts released more donor lymphocytes into the recipient organism than the liver allografts. However, at day + 14, no donor cells could be detected by flow cytometry in either of the three compartments.

Microchimerism

In a next step, we analyzed the persistence of donor cells in peripheral organs of long-term survivors after LTx and LSBTx. Flow cytometry failed to provide reliable

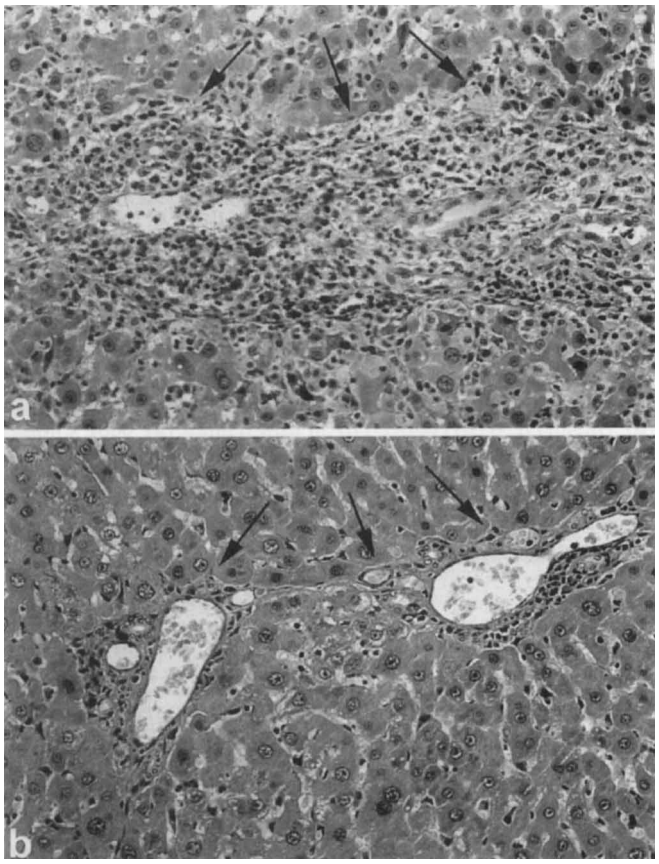

Fig. 2 BN-to-LEW liver allografts after liver/small bowel transplantation, H&E histology, magnification × 200 (**a**) day + 14 and (**b**) day + 100 (*arrows* portal triads)

data in the three compartments after day + 7 due to a lack of sufficient cell numbers. Thus, immunohistochemistry of the spleen – as a representative lymphoid organ – was chosen to detect the cells in question for both groups. To differentiate donor cells from the recipient's parenchyma, monoclonal antibodies directed against the donor type (BN) MHC class I molecules (mAb O×27) were used. Immunohistochemistry clearly demonstrated a small but persisting donor cell population starting from day + 7 up to day + 100 after LTx and LSBTx (Fig. 3). The kinetics of this cell population neither showed correlation with the kinetics of the transient rejection crises in both groups, nor with the processes leading to long-term acceptance.

Graft chimerism

Beside the recipients' spleens, we analyzed chimerism within the allograft compartment itself by immunohistochemistry. Allografts of all groups showed a typical recipient-derived leukocyte infiltration in the portal triads

Table 2 Survival after donor-specific *(BN)* and third-party *(DA)* heart transplantation in LEW recipients secondary to BN liver or BN liver/small bowel allografts

1st allograft	2nd allograft	Survival time, 1st allograft (days)	Survival time, 2nd allograft (days)
BN liver	BN heart	> 100 (3 ×)	> 100 (3 ×)
BN liver	DA heart	> 100 (3 ×)	6, 7, 7
BN liver/ small bowel	BN heart	> 100 (3 ×)	> 100 (3 ×)
BN liver/ small bowel	DA heart	> 100 (3 ×)	7, 7, 8

and parenchyma of the liver, and in the crypts of the small bowel, respectively. This occurred mostly during the first 2 post-operative weeks, indicating an acute cellular rejection process. The recipient-derived leukocyte infiltrate consisted mainly of $CD8^+$ T cells and macrophages with a higher infiltration density in the small bowel allograft after SBTx than after LSBTx. This corresponded with the more serious rejection and cell damage after isolated SBTx. Quite unexpectedly however, donor-derived leukocytes survived the rejection crisis in the liver parenchyma, particularly in the sinusoids (Fig. 4). The kinetics of donor- and recipient-derived leukocyte populations in the sinusoids from day + 7 until day + 100 are demonstrated in Fig. 5: the recipient-derived population increased transiently during the rejection crisis until day + 14, mostly as a consequence of $CD8^+$ T effector cells, and decreased continuously thereafter until day + 100. In contrast, the donor-derived population decreased significantly from day + 7 until day + 14 and increased during tolerance induction until day + 100. This specific cell migration pattern was much more pronounced after LTx than after LSBTx.

Differentiation of donor leukocytes in the sinusoids of the liver allograft

In accordance with previous findings, the number of donor-derived leukocytes along the liver sinusoids changed with rejection and graft acceptance after LTx and LSBTx. Double staining immunohistochemistry was applied to differentiate these leukocytes in α/β T cells, dendritic cells, and Kupffer cells. Donor α/β T lymphocytes were detected not only in the portal triads, but also in the sinusoids at days + 7 and + 14. At day + 100 following LTx and LSBTx however, donor α/β T cells were entirely restricted to the sinusoidal area. Donor-derived dendritic cells (DCs) could be demonstrated clearly in the portal triads and in the sinusoids of the liver allograft during the early postoperative period. Strikingly, donor-derived DCs continued to exist at day + 100

Table 3 Flow-cytometric detection of donor lymphocyte populations in blood, spleen, and mesenteric lymph nodes *(MLNs)* of the recipient after allogeneic liver *(LTx)*, small bowel *(SBTx)*, and combined liver/small bowel transplantation *(LSBTx)* ($n = 3$ for each group, values shown as mean)

		Day + 3			Day + 7		
		LTx	SBTx	LSBTx	LTx	SBTx	LSBTx
Blood							
BN^+	B cells	0.09 ± 0.07	0.61 ± 0.30	0.72 ± 0.21	0.33 ± 0.22	0.07 ± 0.03	0.87 ± 0.26
	$CD4^+$ T cells	0.48* ± 0.17	5.07 ± 1.10	3.64 ± 1.08	0.46 ± 0.15	0.03** ± 0.01	0.29** ± 0.15
	$CD8^+$ T cells	0.09 ± 0.03	0.41 ± 0.20	0.27 ± 0.14	0.16 ± 0.07	0.01 ± 0.01	0.15 ± 0.06
Spleen							
BN^+	B cells	0.45 ± 0.15	1.57 ± 0.50	2.86 ± 0.50	0.55 ± 0.08	0.25 ± 0.04	0.51 ± 0.06
	$CD4^+$ T cells	0.45* ± 0.18	4.92 ± 1.40	4.25 ± 0.74	0.41 ± 0.26	0.11** ± 0.07	0.18** ± 0.08
	$CD8^+$ T cells	0.14 ± 0.04	0.71 ± 0.10	0.55 ± 0.19	0.42 ± 0.20	0.04 ± 0.05	0.15 ± 0.03
MLNs							
BN^+	B cells	0.13 ± 0.03	1.90 ± 1.01	0.94 ± 0.14	0.64 ± 0.08	0.24 ± 0.07	0.95 ± 0.34
	$CD4^+$ T cells	0.15* ± 0.11	4.93 ± 1.09	2.01 ± 0.70	0.26 ± 0.10	0.07** ± 0.01	0.02** ± 0.14
	$CD8^+$ T cells	0.06 ± 0.02	0.36 ± 0.21	0.17 ± 0.06	0.15 ± 0.07	0.06 ± 0.04	0.07 ± 0.04

* $P < 0.0001$ LTx vs LSBTx, SBTx
** $P < 0.0001$ day + 3 vs day + 7)

in the liver allografts of both experimental groups (Fig. 6). At that time, recipient-derived DCs were predominantly found within the portal triads (90 ± 7% recipient DCs vs 10 ± 7% donor DCs). In contrast, the sinusoids were populated by only 37 ± 10% recipient-derived DCs vs 63 ± 10% donor DCs. An infiltration of recipient-derived Kupffer cells (KCs) was observed in the liver allograft, but at day + 100 after LSBTx nearly half of the KCs were of donor origin (53 ± 8% donor vs 47 ± 8% recipient KCs).

Discussion

The value of chimerism for allograft survival is discussed controversially in the respective literature [37, 44]. The major arguments focus on two points: on the number and on the type of donor cells that are detected in the recipient at different points in time after transplantation.

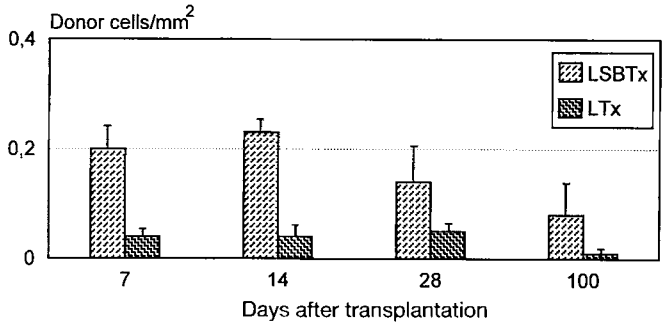

Fig. 3 Donor cell population in the spleen at days + 7, + 14, + 28, and + 100 after liver *(LTx)* and liver/small bowel transplantation *(LSBTx)* ($n = 3$ for each group, values shown as mean), determined as mAb O × 27^+ cells by immunohistochemistry

Macrochimerism

The data obtained from our experiments illustrate macrochimerism in the recipient's blood and spleen following the 1st week after SBTx and LTx. Comparing these two models, the small bowel allograft carries significantly more T and B lymphocytes into the recipient than the liver allograft (Table 3). This cell invasion is followed by a stronger rejection response in the small bowel recipient, eliminating donor cells from the recipient's blood. In the case of LSBTx, the large number of donor cells obviously originates from the co-transplanted small bowel, yet cell numbers decrease after the 1st postoperative week to the lower level observed after LTx. In conclusion, macrochimerism is induced on a large scale after small bowel transplantation (alone or in combination with the liver), which does not, however, lead to a better graft acceptance.

These data are in clear contrast to observations made with respect to monkeys: here a minimum level of macrochimerism (> 1.5% donor leukocytes in the recipient's blood) appears to be essential for a long-term acceptance of a kidney allograft [15]. But in this particular experimental setting, kidneys were transplanted simultaneously with bone marrow under immunosuppression. Most likely, the donor cells found in the recipient originated in the cotransplanted bone marrow. Comparing the levels of macrochimerism reached after liver [47], bone marrow [17], kidney [15], or small bowel transplantation [19], we conclude that the phenomenon depends on both, the type of allograft and the strength

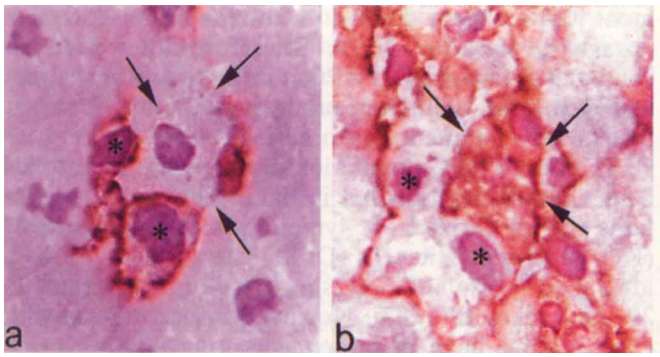

Fig. 4 Sinusoidal cells in the liver allograft at day + 100 after combined liver/small bowel transplantation in serial sections, magnification × 1000: (**a**) stained with mAb NDS60 (LEW, recipient), (**b**) stained with mAb O×27 (BN, donor) (*arrows* donor cells, *asterisks* recipient cells)

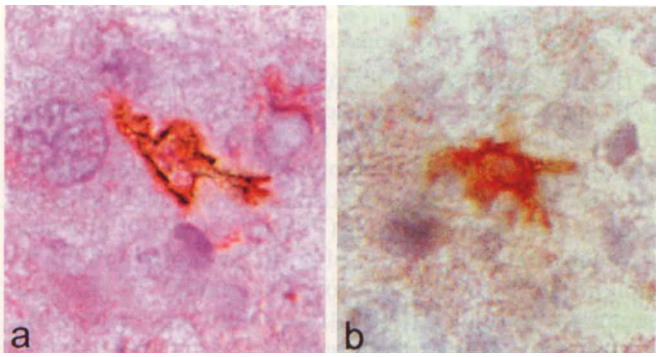

Fig. 6 Dendritic cells in the liver sinusoids at day + 100 after liver/small bowel transplantation (*LSBTx*): (**a**) recipient dendritic cell, (**b**) donor dendritic cell. Double staining immunohistochemistry (magnification × 1000): *brown* (peroxidase): mAb O×62 (dendritic cells); *red* (Fast red): mAb O×27 BN, donor)

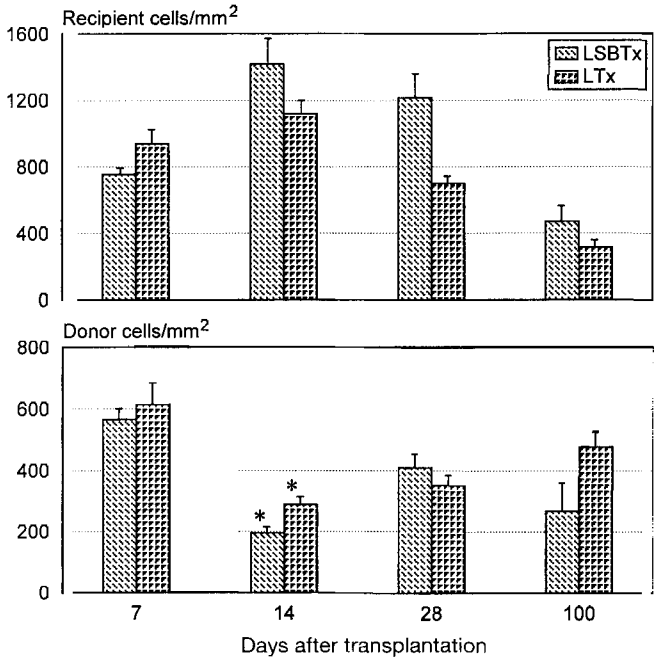

Fig. 5 Recipient (mAb NDS60$^+$) and donor (mAb NDS60$^-$) cell population in the sinusoids of liver allografts at days + 7, + 14, + 28, and + 100 after liver (*LTx*) and liver/small bowel transplantation (*LSBTx*) ($n = 3$ for each group, values shown as mean, $^*P < 0.005$ vs day + 7)

of the recipient's immune response. This immune response, on the other hand, is markedly influenced by different immunosuppressive protocols that are used during organ transplantation [24]. If macrochimerism depends on these two conditions, it appears to be a highly unsuitable parameter to predict long-term allograft acceptance.

Microchimerism

Due to the small numbers of donor cells, flow cytometry failed to detect these cells in the recipient's periphery 100 days after LTx and LSBTx. However, immunohistochemistry demonstrated donor cells in the recipient's spleen (Fig. 3). At present, it is difficult to assess whether these cells survive the transient rejection response or originate from hematopoietic stem cells inside the donor organ [23], which may constantly release these cells into the recipient's periphery. The capacity of liver stem cells to produce mature blood lymphocytes is demonstrated clearly in lethally irradiated animals that survived after liver transplantation [46]. Additional observations were made in tolerant liver recipients, with whom removal of the liver not only resulted in a loss of tolerance but in a loss of the peripheral donor cell population as well [12, 25]. The fact that the numbers of peripheral donor cells show only little variation during rejection and tolerance (Fig. 3) supports our doubts that microchimerism can serve as a useful indicator for tolerance induction. These doubts are shared by other investigators: human liver allografts have been rejected in the presence of peripheral donor cells [22, 30], and rat or mouse liver allografts have been accepted in the long-term without any detectable donor cell population in the recipient's periphery [2, 8, 33].

Neither macrochimerism nor microchimerism therefore appear to be valuable parameters to predict the immune response of the recipient. We believe that the allograft determines the presence of peripheral donor cells rather than being influenced itself by their existence.

Graft chimerism

If the allograft itself determines the level of chimerism [24], the question is which cell population is responsible for this phenomenon and – in particular in the liver allograft -modulates the recipient's immune response towards tolerance. After transplantation, each allograft contains parenchymal cells (PCs) of the donor and nonparenchymal cells (NPCs) of both, the donor and the recipient. In particular the immunogenicity of the liver seems to be reduced after transplantation because donor-type NPCs have been replaced by recipient cells of similar phenotype [31]. Retransplantation of such a "modified" liver allograft (donor PCs, recipient NPCs) into a naive recipient induces tolerance again. If this exchange process were complete, these experiments would certainly underline the role of donor PCs in tolerance induction [35]. However, our data produce little support for this theory: even in tolerized liver allografts at 100 days after LTx and LSBTx, donor NPCs were found (Fig. 5). Donor and recipient NPCs persist in the liver, establishing a special kind of graft chimerism that is first noticed in this particular setting. Compared to the results after LSBTx, the development of graft chimerism in the liver was more pronounced after LTx. A possible explanation is that the more moderate rejection episode results in a smaller decrease of donor NPCs, and that the earlier recovery of the liver tissue during tolerance leads to a higher donor PC population after LTx (Fig. 5). The kinetics of the donor NPC population in the liver suggest a connection between this process and the changing immune response of the recipient.

Research in the field of chimerism was so far focused on the recipient's periphery [20, 26, 34]. Recently, however, the degree of microchimerism has been related to the cell migration process in the allograft itself. In particular the number of progenitor cells as part of the donor NPC population in the liver allograft seems to be strongly related to the pattern of donor cells in the recipients' bone marrow [28]. In accordance with the data of the current study, the observed kind of graft chimerism in the liver apparently determines the degree of microchimerism seen in the recipient's periphery. In contrast to these findings in the liver, donor NPCs in the small bowel allograft have only been found under immunosuppression [5]. The small bowel contains its own lymphatic system (gut-associated lymphoid tissue, GALT). It is a matter of controversy whether the cell exchange in the GALT is part of a normal process or characterizes a rejection crisis [4, 11]. From the specific point of view of graft chimerism, the relevance of this cell exchange for the recipient's immune response remains uncertain. A rejection-free status for an isolated small bowel allograft cannot be achieved without immunosuppression. Even then, the presence of donor- or recipient-type NPCs in the GALT or their fibrous replacement [16] seems to depend on the immunosuppressive dosage given after SBTx. Therefore, the observed course of donor-derived NPCs during liver transplantation in absence of immunosuppressive therapy seems to be a process unique to this organ.

The donor NPC population in the liver allograft has been characterized further in the present study: it contains donor-derived T lymphocytes, Kupffer cells (KCs) and dendritic cells (DCs). The exchange of KCs after liver transplantation is described for allografts [9, 39] and xenografts [45]. However, in accordance with Julie [13] we have identified persisting donor KCs in the liver allografts of long-term surviving animals after LTx and LSBTx. The half-life of KCs is limited (12.4 days) [42]. Taking into account that donor KCs can be detected 100 days after liver transplantation, they most likely originate from donor-derived stem cells transplanted together with the liver. Donor-derived DCs are also observed in the sinusoids at any time. These observations extend Demetris findings, who in 1992 described the irregular existence of DCs in the liver allograft 30 days after transplantation [6]. Furthermore, the persistence of donor-derived antigen-presenting cells (APCs) in the liver allograft seems to be of specific importance in manipulating the recipient's immune response:

(1) Tolerance after liver transplantation is abolished if these cells are eliminated by irradiating the graft prior to transplantation [32, 38].
(2) Liver allografts and the contained NPCs – but not bone marrow transfusion – prevent chronic rejection in a BN-to-LEW heart transplantation model [7].

These data suggest that the direct alloantigen presentation of donor-derived DCs and KCs may play a major role in the avoidance of immunopathologic processes leading to acute or chronic rejection [40]. The coexistence of these donor APCs together with recipient cells of the same phenotype represents a special kind of graft chimerism. Its kinetics are first described in this study in tolerized liver allografts after LTx and LSBTx.

In conclusion, the degree of macro- and microchimerism in the recipient apparently depends on the type of allograft and its content of passenger leukocytes and hematopoietic stem cells. However, the extent of graft chimerism encountered in the liver allograft, namely the coexistence of donor- and recipient-derived alloantigen-presenting cells (DCs, KCs), appears to be a unique property of this organ. We recently demonstrated that apoptosis of $CD8^+$ T lymphocytes occurs during tolerance induction after liver transplantation [21]. Compared to other abdominal organs, this also seems to be unique to the liver. Whether the persisting donor APCs in the liver allograft promote this phenomenon needs

to be further investigated: the loss of co-stimulatory factors [18], or CD 40 [1] on the cell surface of these liver APCs are possible strategies for T cell inactivation and apoptosis which may subsequently lead to permanent peripheral tolerance.

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