Transpl Int (2000) 13 [Suppl 1]: S537–S540

S. Löffeler
D. Meyer
C. Otto
H.-J. Gassel
W. Timmermann
K. Ulrichs
A. Thiede

Different kinetics of donor cell populations after isolated liver and combined liver/small bowel transplantation

S. Löffeler, D. Meyer, C. Otto, H.-J. Gassel, W. Timmermann, K. Ulrichs, A. Thiede
Department of Surgery, Experimental Transplantation-Immunology and Microsurgery Unit, University of Würzburg, 97080 Würzburg, Germany
S. Löffler (✉) (e-mail: svenloeffel@hotmail.com)
Avdeling for patologi, Det Norske Radiumhospital, Ullernchauseen 70, Montebello, 0310 Oslo, Norway

Abstract Spontaneous tolerance induction after liver transplantation also supports additional transplants, e.g. a small bowel graft, from the same donor (tolerogenic effect). Chimerism serves as a possible explanation of this phenomenon. Isolated liver (LTx) and combined liver/small bowel transplantation (LSBTx) are compared. LSBTx and LTx were performed in the BN → LEW rat strain combination without immunosuppression. Parenchymal damage during rejection was monitored by sequential standard histology. Donor/recipient populations were identified and further differentiated for immunhistochemical single and double staining. A small number of donor specific leukocytes can be detected on all days in host organs (microchimerism). A significantly larger donor leukocyte population survives long-term in the sinusoids of liver (graft chimerism). Sinusoidal donor leukocytes survive rejection and recover in number after tolerance induction. Rejection of liver allografts and infiltration by host leukocytes are more pronounced after LSBTx than after LTx. Accordingly, during rejection a steeper decline of sinusoidal donor leukocytes is observed after LSBTx and recovery after tolerance induction is not as marked. Microchimerism apparently plays no significant role in either transplantation model. The number of sinusoidal donor leukocytes, however, mirrors closely host immune responses.

Key words Chimerism · Graft chimerism · Tolerogenic effect of liver grafts

Introduction

Rejection of transplants appears to be ameliorated by simultaneous liver transplantation from the same donor [10, 16]. Even very immunogenic small bowel grafts benefit from this tolerogenic effect of a liver allograft [6]. The exact mechanism of tolerance induction and the origins of the tolerogenic effect remain unknown, but persistence of donor leukocytes in host peripheral organs (chimerism) is debated as a possible cause [14]. We investigated the role of chimerism in host peripheral organs and liver allografts during rejection and tolerance after isolated liver transplantation (LTx) and combined liver/small bowel transplantation (LSBTx). The aim of this study was to follow the development of donor/recipient leukocyte populations in host organs and liver grafts and to correlate the findings to the rejection response after LTx and LSBTx.

Materials and methods

LSBTx and LTx were performed in allogeneic Brown Norway (BN) → Lewis (LEW) and syngeneic LEW → LEW rat strain combinations following previously published methods [3, 5]: donor intestinal grafts were placed heterotopically. Recipient intestines maintained physiological location and function. Liver grafts were arterialized and portal venous drainage of small bowel grafts was left unaltered. Heterotopic heart transplantion from either BN or

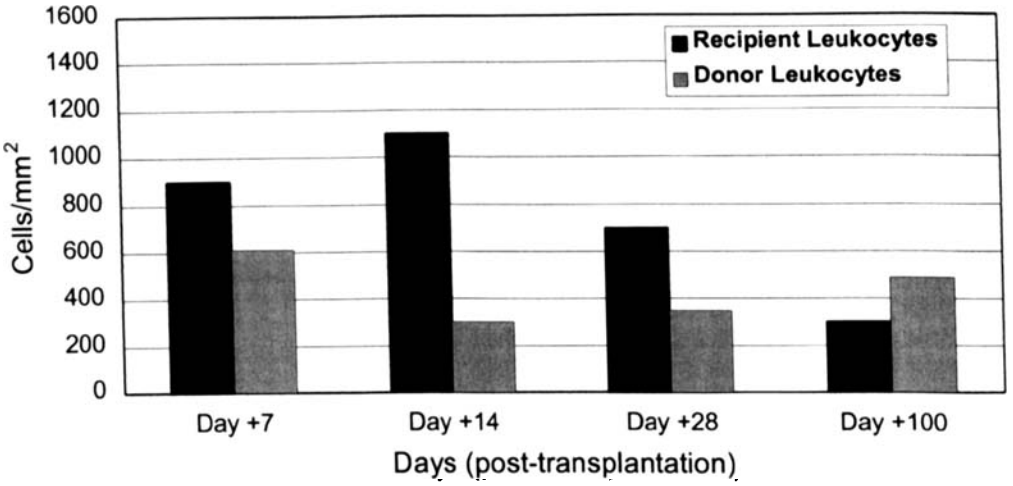

Fig. 1 Development of donor and recipient sinusoidal leukocyte populations after allogeneic liver transplantation (*LTx*)

Dark Agouti (DA) donors + 70 days after LSBTx was performed to verify tolerance induction.

Animals received no immunosuppressive treatment and were killed on days + 7, + 14, + 28 and + 100. Transplants and recipient spleens were fixed in buffered formalin and paraffin sections were stained with hematoxylin and eosin (H&E) following standard protocols. H&E histology sections were evaluated according to international criteria for monitoring of rejection of human liver grafts [2] modified for application to rat liver transplants. Frozen sections of the same organs were required for immunhistochemical staining following previously published protocols [7]. Mabs Ox27 (BN-specific, Serotec, UK) and NDS60 (LEW-specific, Dallman MJ, UK) served to identify donor and recipient populations. Evaluation was performed by counting + cells/mm^2 tissue section. Further differentiation of donor cell populations + 100 days after LSBTx was achieved by using a double staining technique involving two primary antibodies: Ox62 (dendritic cells), ED2 (Kupffer cells) and RT73 (α/β-T-cells), respectively (Serotec) were combined with mAb Ox27. The first primary mAb was processed following the same protocol as for single staining (see above). The second primary mAb was first incubated with linked solution [Biotin-conjugated F(ab')-fragment-IgG, donkey-anti-mouse, Jackson Immuno Research, USA] and consequently with label-solution (alkaline phosphatase-linked streptavidin, Super Sensitive Detection Kit, Bio Genex, USA). Signal convertion was achieved using Fast-red substance (Bio Genex).

Results

Eighty percent of recipients after LTx survive long-term and develop graft tolerance, while this holds true for only 70% of animals after LSBTx. Tolerance is verified by rejection of third party hearts (DA) and acceptance without rejection of donor specific cardiac allografts (BN) transplanted heterotopically + 70 days after LSBTx. Animals after LTx do significantly better on clinical presentation (e.g. increase in body weight) than their counterparts after LSBTx. Transient rejection of liver allografts based on H&E histology is considerably more pronounced and prolonged after LSBTx. Infiltration of liver grafts by host cells (NDS60 +) after LSBTx occurs on a larger scale during rejection and decrease of the infiltration in the late stages of rejection is much slower (see Figs. 1 and 2). Size of donor leukocyte populations (Ox27 +) in the liver grafts correspondingly decrease further after LSBTx than after LTx (see Figs. 1 and 2). At the end of rejection, portal areas of the liver allografts after LTx and LSBTx are almost completely depleted of donor leukocytes while the development of sinusoidal donor leukocyte populations takes a different course: depletion of sinusoidal donor cells on the height of rejection is incomplete. This population even increases again after tolerance induction. This process eventually leads to the point where + 100 days after LTx donor leukocytes represent the majority of the sinusoidal leukocyte population. Sinusoidal donor cells after LSBTx also recover after tolerance induction but not as profoundly. Further differentiation of sinusoidal donor populations + 100 days after LSBTx based on immunhistochemical double staining shows that the majority of sinusoidal donor leukocytes are Kupffer cells. However, donor dendritic cells and T-cells also survive long-term in the sinusoidal environment (data not shown).

Frozen sections from host spleens are used to detect donor cells (Ox27 +) in the host organism (microchimerism). Results show that donor cell populations in peripheral host organs are minute as compared to sinusoidal donor populations (between 0.25 and 0.02 cells/mm^2). Development of this population shows no significant changes during transient rejection and tolerance induction.

Discussion

Persistence of donor leukocytes in the host organism (microchimerism) is frequently observed after organ transplantation [$\neq$ 4]. The importance of microchimer-

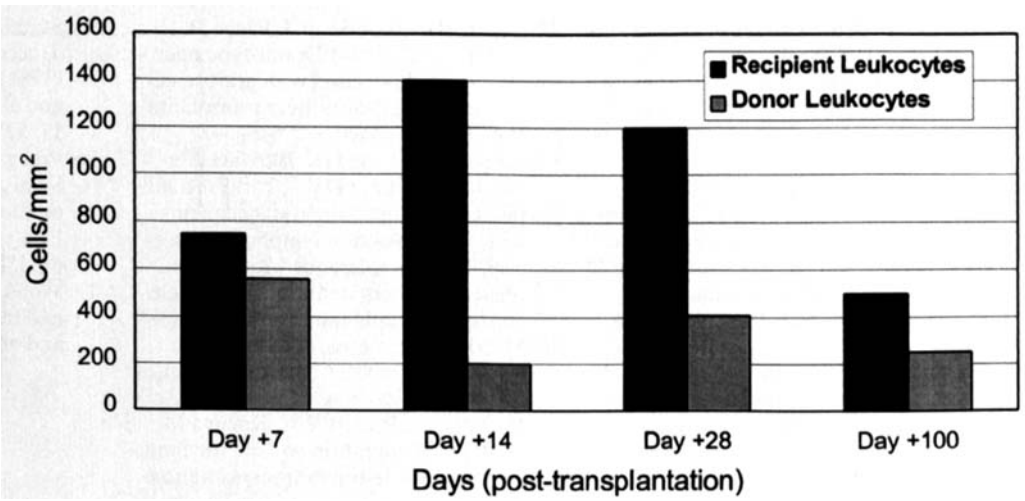

Fig. 2 Development of donor and recipient sinusoidal leukocyte populations after allogeneic combined liver small bowel transplantation (*LSBTx*)

ism for organ engraftment is still debated [15, 17]. Rejection of transplants has been reported despite established chimerism [8, 11, 12]. Transplants on the other hand are accepted without detectable chimerism [1, 4, 13]. These reports have cast some doubt on the indispensability of microchimerism for organ engraftment. In our study, microchimerism could be detected on all days in long-term survivors after LTx and LSBTx. There was neither a significant difference between LTx and LSBTx nor was there any correlation between microchimerism and the immune status of the host: the number of cells underwent no significant changes during rejection and tolerance induction.

Contrary to these findings, a large number of donor leukocytes survived long term in the sinusoids of the liver grafts. This contradicts previous publications, which claimed a complete exchange of donor leukocytes by host cells [19]. There was also a striking correlation between the status of the host immune system and the development of this population. The stronger and prolonged rejection response after LSBTx was mirrored by a sharper decline and a slower recovery of the donor population after tolerance induction.

These findings open a wide range of new experimental approaches to liver transplantation. A resident sinusoidal leukocyte population which is able to survive rejection by the host immune system holds the potential for manipulation in vivo before explantation or in vitro after explantation prior to transplantation. Manipulation may include, e. g. confrontation with recipient antigen in general or the host immune system, in particular before transplanting the liver graft.

The results justify an intensified investigation of chimerism in liver grafts. Microchimerism on the other hand seems to play a minor role for tolerance induction after LSBTx.

References

1. Bushell A, Pearson T, Morris PJ, Wood KJ (1995) Donor-recipient microchimerism is not required for tolerance induction following recipient pretreatment with donor-specific transfusion and anti-CD4-antibody. Transplantation 59: 1367–1371
2. Demetris AJ, Murase N, Ye Q, Galvao FHF, Richert C, Saad R, Pham S, Duquesnoy RJ, Zeevi A, Fung JJ, Starzl TE (1997) Analysis of chronic rejection and obliterative arteriopathy – possible contributions of donor antigen-presenting cells and lymphatic disruption. Am J Pathol 150: 563–578
3. Engemann R (1993) Orthotopic liver transplantation in the rat. Langenbecks Arch Chir 378: 66–67
4. Fisher RA, Cohen DS, Ben-Ezra JM, Sallade RE, Tawes JW, Tarry WC (1996) Induction of long-term graft tolerance and donor/recipient chimerism. J Surg Res 60: 181–185
5. Gassel HJ, Timmermann W, Meyer D, Gassel AM, Thiede A (1997) Investigations of the immunoprotective role of the liver after allogeneic orthotopic combined liver-small bowel transplantation. Transplant Proc 29: 693
6. Meyer D, Gassel HJ (1998) Combined abdominal organ transplantation. In: Timmermann W, Gassel HJ, Ulrichs K, Zhong R, Thiede A (eds) Organ transplantation in rats and mice, microsurgical techniques and immunological principles. Springer Verlag, Heidelberg, pp 141–147
7. Meyer D, Baumgardt S, Löffeler S, Czub S, Otto C, Gassel H-J, Timmermann W, Thiede A, Ulrichs K (1998) Apoptosis of T lymphocytes in liver and/or small bowel allografts during tolerance induction. Transplantation 66: 1530–1536
8. Molleston JP, Alevy YG, SivaSai KS, Mohanakumar T, Howard TK (1996) Evidence that pediatric liver transplant recipients may undergo late rejection episodes in spite of donor-specific microchimerism. Transplantation 61: 656–657

9. Murase N, Demetris J, Matsuzaki T, Yagihashi A, Todo S, Fung J, Starzl TE (1991) Long survival in rats after multivisceral versus isolated small bowel allotransplantation under FK506. Surgery 110: 87–98
10. Rasmussen A, Davies HS, Jamieson NV, Evans DB, Calne RY (1995) Combined transplantation of liver and kidney from the same donor protects the kidney from rejection and improves kidney graft survival. Transplantation 59: 919–921
11. Schlitt HJ (1997) Is microchimerism needed for allograft tolerance? Transplant Proc 29: 82–84
12. Schlitt HJ, Hundrieser J, Ringe B, Pichlmayr R (1994) Donor-type microchimerism associated with graft rejection eight years after liver transplantation. N Engl J Med 330: 646–647
13. Shirwan H, Wang HK, Barwari, Makowka L, Cramer DV (1996) Pretransplant injection of allograft recipients with donor blood or lymphocytes permits allograft tolerance without the presence of persistent donor microchimerism. Transplantation 61: 1382–1386
14. Starzl TE, Demetris AJ, Trucco M, Murase N, Ricordi C, Ildstad S, Ramos H, Todo S, Tzakis A, Fung JJ, Nalesnik M, Zeevi A, Rudert WA, Kocova M (1993) Cell migration and microchimerism after whole-organ transplantation: the basis of graft acceptance. Hepatology 17: 1127–1152
15. Starzl TE, Demetris AJ, Murase N, Trucco M, Thomson AW, Rao AS (1996) The lost cord: microchimerism and allograft survival. Immunol Today 17: 577–584
16. Wang C, Sun J, Li L, Wang L, Dolan P, Sheil AG (1998) Conversion of pancreas allograft rejection to acceptance by liver transplantation. Transplantation 65: 188–192
17. Wood K, Sachs DH (1996) Chimerism and transplantation tolerance: cause and effect. Immunol Today 17: 584–587