Liver transplantation : chimerism, complications and matrix metalloproteinases

Hove, W.R. ten

Chapter 2

Extensive chimerism in liver transplants: Vascular endothelium, bile duct epithelium, and hepatocytes

W. Rogier ten Hove
Bart van Hoek
Ingeborg M. Bajema
Jan Ringers
Johan H.J.M. van Krieken
Emma L. Lagaaij

Liver Transplantation 2003;9:552-556.

Abstract

Background

The transplanted liver has been shown to be particularly capable of inducing tolerance. An explanation may be the presence of chimerism. Cells of donor origin have been found in recipient tissues after transplantation of any solid organ. Evidence for the presence of cells of recipient origin within the transplanted liver is very limited. We investigated whether nonlymphoid cells of recipient origin can be found within human liver allografts.

Methods

Five male patients who received a liver transplant from a female donor and 11 patients who received an HLA-I mismatched liver transplant were studied. We confirmed our observations with two different techniques in combination with double-staining techniques. To identify male cells in female liver transplants, we used in situ hybridiza- tion for sex chromosomes. To identify specific HLA class I antigens of recipient origin, we used immunohistochemistry with HLA class I–specific antibodies. Double staining was performed to discriminate different cell lineages and inflammatory cells.

Results

Endothelial cells of recipient origin were found in 14 of 16 donor livers. Bile duct epithelial cells of recipient origin were found in 5 of 16 cases. Hepatocytes of recipient origin were seen in only 1 of the 5 studied sex-mismatched donor livers.

Conclusion

Our study provides evidence that cells of recipient origin can replace biliary epithelial cells, endothelial cells, and hepatocytes within the human liver allograft.

This is consistent with the concept that circulating pluripotent progenitor cells exist, capable of differentiating into endothelial cells, epithelial cells, and hepatocytes.

Introduction

In the early days of solid-organ transplantation, it was postulated that the success of renal transplantation could be explained by the existence of chimerism within the graft. Especially chimerism of endothelial cells was thought to be relevant because endothelium is one of the major targets for graft rejection. Replacement of donor endothelial cells by recipient cells therefore would reduce the immunogenicity of the graft.[1] During the past four decades, several studies addressed the issue of intragraft chimerism in solid-organ transplants, with conflicting results.[2-7] Most studies did not find chimerism or found it only sporadically in poorly functioning grafts. Therefore, it became generally believed that non- lymphoid cells in organ grafts remain of donor origin. However, we found clear evidence of endothelial cell chimerism in renal allografts, and cardiac chimerism has also been described recently.[8,9]

The liver, in comparison to other transplanted organs, has been shown to be particularly capable of inducing tolerance.[10-13] A number of hypotheses have been put forward to explain this immune-privileged state. One of these is the presence of chimerism.[14-16] Other factors mentioned are regenerative capacity and the production of soluble major histocompatibility complex. Many studies addressed the issue of chimerism outside the graft resulting from donor-derived highly immunogenic passenger leukocytes, of which the liver is particularly rich. Donor-derived cells can be found in recipient peripheral tissues years and even decades after transplantation.[14,17] The clinical relevance of the persistence of donor leukocyte chimerism is still unclear.[18]

Little is known about chimerism within the human liver allograft. Only few have studied this human hepatic intragraft chimerism.[19,20] For decades, it has been the general belief that only Kupffer cells of recipient origin can be found within the transplant, whereas endothelial cells, bile duct epithelial cells, and hepatocytes remain of donor origin.[19] In animal studies, evidence is growing that bonemarrow–derived stem cells can differentiate into various hepatic cell types, such as hepatocytes and endothelial cells.[21-24] We therefore investigated whether nonlymphoid cells of recipient origin can be found within human liver allografts.

Patients and Methods

Patients and Biopsy Specimens

Five male patients who received a liver transplant from a female donor were selected. None of the female donors had had male offspring. In addition, 11 patients with HLA-I mismatching allografts for A2, A3, A9, or A11 were studied. All patients had undergone orthotopic liver transplantation at Leiden University Medical Center (Leiden, The Netherlands) between 1993 and 1998. Liver biopsy samples were obtained 1 year after transplantation, according to protocol. Additional biopsy specimens obtained early after transplantation were studied of one selected patient with evidence of extensive chimerism in the 1-year biopsy specimen. As per protocol, a part of every biopsy specimen had been stored at 80°C, whereas the other part was formalin fixed and stored in paraffin.

Approval by the Ethics Committee of Leiden University Medical Center was obtained.

HLA Typing

HLA typing for antigens of class I was performed using standard serological methods by complement-dependent microcytotoxicity on peripheral-blood leukocytes of recipients and splenocytes of donors, which are available as part of the routine pretransplantation workup.

In Situ Hybridization Sex Chromosomes

For sex-chromosome identification in the five sex-mismatched grafts, we performed in situ hybridization using repetitive DNA probes specific for X and Y chromosomes, as previously described.[25] Briefly, probes were biotinylated by nick translation and dissolved in a 60% formamide hybridization mixture. Paraffin sections 6-mm thick were cut and mounted on poly-L-lysine–coated slides. Predigestion steps consisted of incubation in 1 mol/L of sodium thiocyanate solution at 80°C, followed by 60 to 90 minutes of treatment with 0.5% pepsin in 0.1 mol/L of hydrochloric acid. Hybridization was performed overnight at 42°C. The hybridization reaction was visualized with avidin, biotinylated goat antiavidin, and avidin-peroxidase developed with diaminobenzidine. Positive and negative controls for in situ hybridization were biopsy specimens from normal male and female livers.

Immunohistochemical Analysis

For HLA class I antigens, immunohistochemical staining was performed

on cryostat sections from the 11 HLA-I–mismatched grafts, as previously described.[26] In short, sections were fixed in cold acetone and incubated with the primary antibody. Four monoclonal antibodies were used that recognize the HLA class I antigens A2, A3, A9, and A11 (American Type Culture Collection, Rockville, MD). A two-step immunoperoxidase technique was used with 3-amino-g-ethyl-carbazol as a coloring substrate. Each patient was tested with all antibodies, which provided many positive and negative controls. For additional negative controls, the second antibodies were replaced by phosphate-buffered saline. Recipient-derived graft-infiltrating cells stained positive for recipient major histocompatibility complex antigens and thus served as internal positive controls.

Additional Staining Techniques

For double staining, antibodies against endothelial cell–specific antigens (CD31, factor VIII; Dako, Carpinteria, CA) against lymphocytes, monocytes, and other inflammatory cells (CD45-LCA; Dako) and bile duct epithelial cells (keratin 18; LUMC, Leiden, The Netherlands) were combined with immunohistochemistry with HLA class I–specific antibodies and in situ hybridization with sex chromosomes. When possible, double staining was realized on the same slide. In other cases, different staining techniques were performed on consecutive slides.

Results

In Situ Hybridization for X and Y Chromosomes

Endothelial cells of recipient origin, i.e., Y chromosome positive, were found in all five sex-mismatched patients. A detail of a biopsy sample of a female liver transplanted into a male recipient can be seen in Figure 1A. This detail of a vessel wall shows an endothelial cell staining positive with the Y chromosome probe, indicating male (recipient) origin. Hepatocytes of recipient (male) origin were seen in only one of five studied patients, shown in Figure 1B. Most hepatocytes stain negative, indicating the donor origin of these cells, but some contain a Y chromosome. Hepatocytes can be tetraploid, which explains why two spots sometimes can be seen within one cell. Partial nuclear sampling in tissue sections may lead to undercounting of Y-positive nuclei. Recipient-derived bile duct epithelial cells were seen in three of five patients. Figure 1C shows a biopsy sample of a female liver graft transplanted into a male recipient 1-year posttransplantation, showing a bile duct. Epithelial cells containing a Y chromosome can be seen,

indicating the presence of male epithelial bile duct cells of recipient origin. Double staining with periodic acid–Schiff (PAS) makes the bile duct stand out because bile duct epithelial cells are PAS negative. Figure 1D shows the same bile duct in a consecutive slide with CD45 staining to confirm that the duct is free of inflammatory cells.

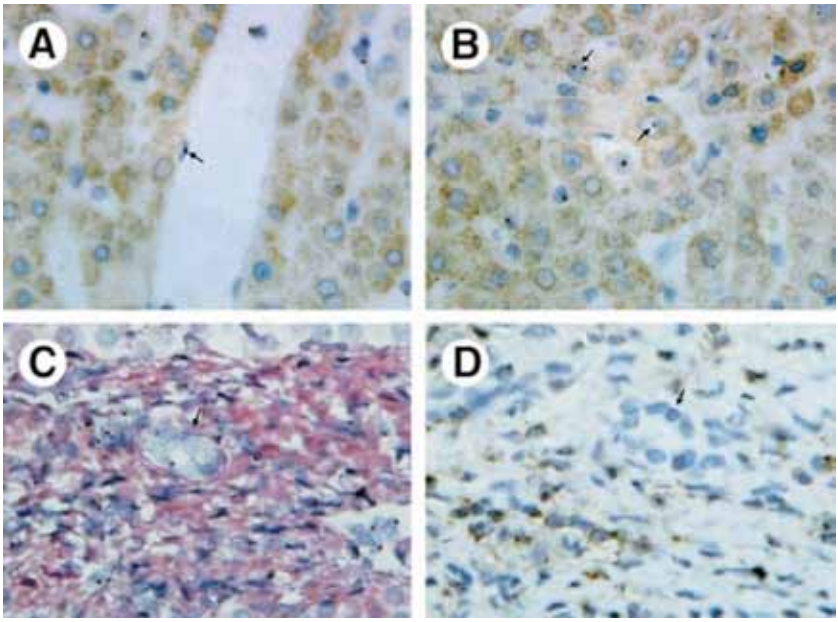

Figure 1.
In situ hybridization for Y chromosomes. A female donor liver was transplanted into a male recipient. In situ hybridization with a Y-specific centromeric DNA probe was performed on a posttransplantation biopsy sample. In (A) endothelial cells of middle large vessels, (B) hepatocytes, and (C, D) bile duct epithelial cells, clear signals can be appreciated after in situ hybridization with this probe, indicating that chimerism has taken place in all three cell types. (C) A PAS background that makes the bile duct stand out because cholangiocytes are PAS negative. (D) Staining on a consecutive slide with CD45 and no inflammatory cells can be seen in or near the bile duct. The female donor of this graft had no male offspring. The presence of endogenous biotin in hepatocytes causes some background staining. (Original magnification X400.)

Immunohistochemistry for HLA Class I Antigens

A biopsy sample of an HLA-A2–negative liver graft transplanted into an HLA-A2–positive recipient is shown in Figure 2. A bile duct with biliary epithelial cells staining positive for recipient type HLA-A2 is shown in Figure 2A. This chimerism of bile duct epithelial cells was observed in 2 of these 11 HLA-mismatched patients and could be seen in smaller, as well as larger, bile ducts. Replacement of donor type vascular endothelium by recipient type could be observed to a variable degree in 9 of the 11 studied patients. Sinusoidal endothelium staining positive for recipient-

type HLA-A2 is shown in detail in Figure 2B, with double staining with CD31, which stains endothelial cells irrespective of their origin. Hepatocytes were difficult to differentiate with certainty from inflammatory cells with this technique and therefore were not scored in this series.

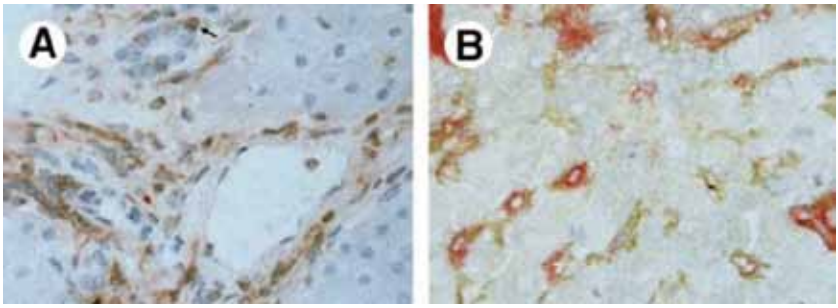

Figure 2.
Staining with recipient-type HLA antibody. An HLA-A2-negative liver was transplanted into an HLA-A2-positive recipient. Immunohistochemical staining against the HLA type of the recipient (HLA-A2) was performed on a biopsy sample obtained 1 year posttransplantation. (A) Bile duct epithelial cells stain positive for recipient type HLA-A2. (B) Sinusoidal endothelium staining positive for recipient type HLA-A2 is shown in red, and double staining against factor VIII is shown in brown. (Original magnification X 400.)

To investigate whether chimerism can develop earlier than 1 year after transplantation, we studied serial liver biopsy specimens by HLA staining from a patient in whose graft we found extensive endothelial cell chimerism 1 year after transplantation. We found no evidence for chimerism in the biopsy sample obtained 1 week after transplantation, whereas it became apparent in the specimens obtained 3 months after transplantation.
One male HLA-A3–negative patient received an HLA-A3–positive graft from a female donor. In this patient, we used both in situ hybridization for Y chromosomes and immunohistochemistry for HLA-A3. We observed endothelial cell chimerism, but no bile duct epithelial cell chimerism, with both techniques in this patient.

Discussion

Our study provides evidence that cells of recipient origin can replace biliary epithelial cells, endothelial cells, and hepatocytes within the human liver allograft. We confirmed our observations using two different techniques. Additional staining techniques were performed to distinguish different cell lineages from inflammatory cells of recipient

origin. Replacement of biliary duct epithelium was observed in one third of patients (5 of 16 patients with the two techniques combined). Endothelial cell chimerism was found to be very common (14 of 16 patients). Hepatocytes of recipient origin were found in only one of five donor livers studied with in situ hybridization for X and Y chromosomes (the HLA stain is not suitable for looking at hepatocyte chimerism). Chimerism within the human liver transplant to such extent that it involves endothelium, bile duct epithelium, and hepatocytes has not been reported previously. For decades, the general belief has been that lymphocytes and Kupffer cells of recipient origin are found in the liver, but graft bile duct epithelium, hepatocytes, and endothelium were considered to remain of donor origin.[19,27,28]

Gouw et al[19] reported in 1987, with the techniques available at that time, that endothelium, bile duct epithelium, and hepatocytes remained of donor origin. However, recent reports indicate that replacement of donor cells by recipient-derived cells occurs much more frequently than was generally assumed. For instance, Gao et al[20] found male endothelial cells in female liver graft recipients. Similarly, Theise et al[29] reported male hepatocytes and cholangiocytes in female liver grafts recipients; however, Fogt et al[7] recently did not find convincing evidence of stem-cell engraftment into transplanted liver tissue.

Baccarani et al[30] argued that the presence of male cells in female donor livers can result from previous male pregnancies of the donor because this is a verycommon phenomenon in women with male offspring. To date, all published studies mentioned did not provide information concerning pregnancies of the donor. In the present study, we observed Y chromosome–positive cells in grafts from female donors without male offspring (G. Persijn, Eurotransplant Foundation, Leiden, The Netherlands, personal communication, January 2003). In a graft from which serial biopsy specimens were obtained, we did not find endothelial chimerism 1 week after transplantation, whereas it was evident after 3 months. Furthermore, HLA antigens of the recipient are extremely unlikely to be identical to HLA antigens of the offspring of the donor. Our data therefore provide evidence that the observed endothelial cells, bile duct cells, and hepatocytes are of recipient origin and not derived from previous male pregnancies of the female donor.

In the present study, endothelial cell chimerism was much more common than chimerism involving bile duct epithelium. This observation is

consistent with the previously postulated concept that chimerism results from repair of damage, although other causes cannot be excluded.[31]

The liver is very susceptible to vascular damage and, to a lesser extent, bile duct damage caused by ischemia-reperfusion injury and acute cellular rejection.[32,33] This may explain the greater percentage of endothelial chimerism than bile duct epithelial chimerism in the present study. Possibly, apoptotic or necrotic hepatocytes may be replaced mainly by regeneration from a local pool of donor hepatocytes.

Serious damage to biliary ducts can be observed during chronic ductopenic rejection. This complication, which often leads to graft loss, has become a rare condition and was not present in our patients. Although unusual, repair of bile ducts has even been reported in this condition.[34,35] Our findings support the possibility of bile duct repair originating from circulating recipient precursor cells, although this does not exclude that it can occur next to repopulation from a local pool of donor oval cells.

In conclusion, our study provides evidence that recipient-derived cells can replace biliary epithelium, endothelium, and hepatocytes in liver transplants. This is consistent with the concept that circulating progenitor cells exist, capable of differentiating into endothelial and epithelial cells.[21,22]

Contributors

Rogier ten Hove, Malice Lagaaij, Han van Krieken, and Bart van Hoek planned and organized the study, analyzed the data, and wrote the manuscript.
Han van Krieken and Ingeborg Bajema helped design the study and supervised staining techniques. Bart van Hoek initiated collaboration and collected clinical data. Jan Ringers collected data.

References

1. Medawar PB. Transplantation of tissues and organs: Introduction. Br Med Bull 1965;21:97-99.

2. Sinclair RA. Origin of endothelium in human renal allografts. BMJ 1972;4:15-16.

3. Williams GM, ter Haar A, Parks LC, Krajewski CA. Endothelial changes associated with hyperacute, acute, and chronic renal allograft rejection in man. Transplant Proc 1973;5:819-822.

4. Bogman MJ, de Waal RM, Koene RA. Persistent expression of donor antigens in endothelium of long-standing skin xenografts and vulnerability to destruction by specific antibodies. Transplant Proc 1987;19:205-207.

5. Sedmak DD, Sharma HM, Czajak CM, Ferguson RM. Recipient endothelialization of renal allografts. An immunohistochemical study utilizing blood group antigens. Transplantation 1988; 46:907-910.

6. Bittmann I, Dose T, Baretton GB, Muller C, Schwaiblmair M, Kur F, Lohrs U. Cellular chimerism of the lung after transplantation. An interphase cytogenetic study. Am J Clin Pathol 2001; 115:525-533.

7. Fogt F, Beyser KH, Poremba C, Zimmerman RL, Khettry U, Ruschoff J. Recipient-derived hepatocytes in liver transplants: A rare event in sex-mismatched transplants. Hepatology 2002;36: 173-176.

8. Lagaaij EL, Cramer-Knijnenburg GF, van Kemenade FJ, van Es LA, Bruijn JA, van Krieken JH. Endothelial cell chimerism after renal transplantation and vascular rejection. Lancet 2001;357:33-37.

9. Quaini F, Urbanek K, Beltrami AP, Finato N, Beltrami CA, Nadal-Ginard B, et al. Chimerism of the transplanted heart. N Engl J Med 2002;346:5-15.

10. Calne RY, Sells RA, Pena JR, Davis DR, Millard PR, Herbertson BM, et al. Induction of immunological tolerance by porcine liver allografts. Nature 1969;223:472-476.

11. Houssin D, Gigou M, Franco D, Bismuth H, Charpentier B, Lang P, Martin E. Specific transplantation tolerance induced by spontaneously tolerated liver allograft in inbred strains of rats. Transplantation 1980;29:418-419.

12. Starzl TE, Demetris AJ, Murase N, Ildstad S, Ricordi C, Trucco M. Cell migration, chimerism, and graft acceptance. Lancet 1992;339:1579-1582.

13. Murase N, Starzl TE, Tanabe M, Fujisaki S, Miyazawa H, Ye Q et al. Variable chimerism, graft-versus-host disease, and tolerance after different kinds of cell and whole organ transplantation from Lewis to Brown Norway rats. Transplantation 1995;60:158-171.

14. Demetris AJ, Murase N, Fujisaki S, Fung JJ, Rao AS, Starzl TE. Hematolymphoid cell trafficking, microchimerism, and GVH reactions after liver, bone marrow, and heart transplantation. Transplant Proc 1993;25:3337-2244.

15. Bishop GA, Sun J, Sheil AG, McCaughan GW. High-dose/ activation-associated tolerance: A mechanism for allograft toler- ance. Transplantation 1997;64:1377-1382.

16. Meyer D, Loffeler S, Otto C, Czub S, Gassel HJ, Timmermann W, et al. Donor-derived alloantigen-presenting cells persist in the liver allograft during tolerance induction. Transpl Int 2000; 13:12-20.

17. Starzl TE, Demetris AJ, Trucco M, Zeevi A, Ramos H, Terasaki P, et al. Chimerism and donor-specific nonreactivity 27 to 29 years after kidney allotransplantation. Transplantation 1993;55: 1272-1277.

18. Wood K, Sachs DH. Chimerism and transplantation tolerance: Cause and effect. Immunol Today 1996;17:584-587.

19. Gouw AS, Houthoff HJ, Huitema S, Beelen JM, Gips CH, Poppema S. Expression of major histocompatibility complex antigens and replacement of donor cells by recipient ones in human liver grafts. Transplantation 1987;43:291-296.

20. Gao Z, McAlister VC, Williams GM. Repopulation of liver endothelium by bone-marrow-derived cells. Lancet 2001;357: 932-933.

21. Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, Li T, et al. Isolation of putative progenitor endothelial cells for angiogenesis. Science 1997;275:964-967.

22. Petersen BE, Bowen WC, Patrene KD, Mars WM, Sullivan AK, Murase N, et al. Bone marrow as a potential source of hepatic oval cells. Science 1999;284:1168-1170.

23. Theise ND, Badve S, Saxena R, Henegariu O, Sell S, Crawford JM, Krause DS. Derivation of hepatocytes from bone marrow cells in mice after radiation-induced myeloablation. Hepatology 2000;31:235-240.

24. Lagasse E, Connors H, Al Dhalimy M, Reitsma M, Dohse M, Osborne L, et al. Purified hematopoietic stem cells can differentiate into hepatocytes in vivo. Nat Med 2000;6:1229-1234.

25. Kibbelaar RE, Leenheers-Binnendijk CF, Spaande PJ, Kluin PM. Biopsy specimen identification by detection of sex chromosomes: Application of in situ hybridisation. J Clin Pathol 1992; 45:149-150.

26. Lagaaij EL, Cramer-Knijnenburg GF, Van der Pijl JW, Bruijn JA, de Fijter JW, van Krieken JH. Rapid verification of the identity of questionable specimens using immunohistochemistry with monoclonal antibodies directed against HLA-class I antigens. Histopathology 1997;31:284-288.

27. Gassel HJ, Engemann R, Thiede A, Hamelmann H. Replacement of donor Kupffer cells by recipient cells after orthotopic rat liver transplantation. Transplant Proc 1987;19:351-353.

28. Gassel HJ, Otto C, Klein I, Steger U, Meyer D, Gassel AM et al. Persistence of stable intragraft cell chimerism in rat liver allo- grafts after drug-induced tolerance. Transplantation 2001;71: 1848-1852.

29. Theise ND, Nimmakayalu M, Gardner R, Illei PB, Morgan G. Teperman L, et al. Liver from bone marrow in humans. Hepa- tology 2000;32:11-16.

30. Baccarani U, Donini A, Risaliti A, Bresadola F. Replacement of liver venous endothelium. Lancet 2001;357:2137.

31. Pober JS. Is host endothelium a silver lining for allografts? Lancet 2001;357:2-3.

32. Wiesner RH, Ludwig J, Krom RA, Hay JE, vanHoek B. Hepatic allograft rejection: New developments in terminology, diagnosis, prevention, and treatment. Mayo Clin Proc 1993;68:69-79.

33. Natori S, Selzner M, Valentino KL, Fritz LC, Srinivasan A, Clavien PA, Gores GJ. Apoptosis of sinusoidal endothelial cells occurs during liver preservation injury by a caspase-dependent mechanism. Transplantation 1999;68:89-96.

34. Hubscher SG, Buckels JA, Elias E, McMaster P, Neuberger J. Vanishing bile-duct syndrome following liver transplantation—Is it reversible? Transplantation 1991;51:1004-1010.

35. Noack KB, Wiesner RH, Batts K, van Hoek B, Ludwig J. Severe ductopenic rejection with features of vanishing bile duct syndrome: Clinical, biochemical, and histologic evidence for spontaneous resolution. Transplant Proc 1991;23:1448-1451.