1381

Cell migration, chimerism, and graft acceptance

THOMAS E. STARZL ANTHONY J. DEMETRIS NORIKO MURASE
SUZANNE ILDSTAD CAMILLO RICORDI MASSIMO TRUCCO

The chimeric nature of the transplanted liver was first shown in our long-surviving human recipients of orthotopic hepatic allografts in 1969.[1] When liver grafts were obtained from cadaveric donors of the opposite sex, karyotyping studies showed that hepatocytes and endothelium of major blood vessels retained their donor specificity, whereas the entire macrophage system, including Kuppfer cells, was replaced with recipient cells.[2] Where donor cells that had left the liver had gone was unknown, but their continued presence was confirmed by the acquisition and maintenance in recipient blood of new donor-specific immunoglobulin (Gm) types[1,3] and red-blood-cell alloantibodies, if donors with ABO non-identity were used.[4] Davies et al[5] attributed the secretion of new soluble HLA class I antigens of donor type to transplanted hepatocytes. However, these HLA molecules come from bone-marrow-derived macrophages and/or dendritic cells,[6] and probably have the same origin from migrated donor cells as the additional Gm types and red-cell antibodies.

Although this early evidence of systemic mixed allogeneic chimerism was circumstantial, we have recently shown with both anatomical and molecular techniques the presence, in clinically stable patients, of peripherally located donor cells many years after liver replacement. For instance, in patients with type IV glycogen storage disease, a disorder in which an insoluble amylopectin-like polysaccharide accumulates throughout the body because of a deficiency in a branching enzyme, we found resorption of extrahepatic amylopectin after liver replacement.[7] This process could not be explained until the migrated donor cells, which had acted as enzyme couriers, were identified by both HLA monoclonal antibodies (fig 1) and polymerase chain reaction (PCR) studies (fig 2) in the biopsied myocardium and skin of 2 patients, 33 and 91 months after hepatic transplantation.

Recent experiments in rats have shown the timing and extent of seeding from the hepatic allograft to both non-lymphoid and lymphoid organs (fig 3).[8] A similar pattern of distribution was found after successful rat-to-mouse bone-marrow transplantation.[9] This similarity between liver transplantation and bone-marrow transplantation has not been reported before. The prompt development, and then the persistence, of this systemic chimerism may help to explain the resistance of the liver to cellular[10] and humoral[11] rejection, as well as its tolerogenicity to other organs from the same donor.[12]

The chimeric structure of the transplanted liver was thought to be a unique feature of this organ for many years until we identified lymphoid and dendritic cell replacement under FK 506 immunosuppression in rat[13] and human[14] intestinal allografts; a similar finding has been reported in swine.[15] In our experiments with rats, the two-way traffic was the same, irrespective of whether bowel was transplanted alone or as a part of a multivisceral graft that also contained liver, stomach, and pancreas.[13] Replaced donor lymphoid and dendritic cells spread through vascular routes to host lymphoid tissues, creating a state of mixed allogeneic chimerism—free of lethal or even clinically detectable graft-versus-host disease (GVHD) except in special strain combinations in which there is a poorly understood imbalance between the graft and recipient immune systems.[16,17]

In addition, GVHD has been only a minor difficulty in human beings after cadaveric small bowel or multivisceral allotransplantation,[14,18,19] despite the use of histoincompatible donors and the routine development (as with the liver) of mixed allogeneic chimerism. Resistance to GVHD has also been described with mixed allogeneic or xenogeneic chimerism after bone-marrow transplantation.[20] This might be explained by responses of coexisting donor and recipient immune cells, each to the other, causing reciprocal clonal expansion followed by peripheral clonal

ADDRESSES: **Pittsburgh Transplant Institute and the Departments of Surgery** (T. E. Starzl, MD, N. Murase, MD, S. Ildstad, MD, C. Ricordi, MD), **Pathology** (A. J. Demetris, MD), **and Pediatrics** (M. Trucco, MD), **University of Pittsburgh Health Science Center, Pittsburgh, Pennsylvania 15213, USA.** Correspondence to T. E. Starzl, Department of Surgery, 3601 Fifth Avenue, 5C Falk Clinic, University of Pittsburgh, Pittsburgh, Pennsylvania 15213, USA.

Fig 1—Heart (upper) and skin (lower) biopsy samples 33 months after liver transplantation.

Immunoperoxidase stain with monoclonal antibody GSP5.3 that reacts with HLA-B7, which was present in the donor and not the recipient. Rust-coloured cells (numerous in heart, sparse in skin) are from donor (magnification × 500).

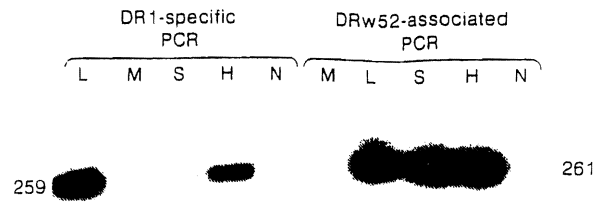

Fig 2—Chimerism after human liver transplantation.

Southern blot analysis of polymerase chain reaction (PCR) products of liver (L), heart (H), and skin (S) 91 months after liver transplantation from HLA-DR1-specific and DRw52-associated amplification of the DRB1 gene in tissue biopsy samples. The presence of the donor DRB1*0101 (donor-specific) allele in all three tissues was confirmed by hybridisation to a DRB-specific probe and by oligonucleotide probe subtyping of DR1-specific PCR products.

M, molecular weight marker; N, PCR-negative control.

deletion (fig 4). If these or similar[21] events do take place, then the deliberate "unbalancing" of the donor-recipient axis by cytoreduction (or cytoablation), which is normally part of bone-marrow transplantation, should be re-examined because it restricts acceptable marrow donors (perhaps unnecessarily) to those with major histocompatibility complex (MHC) concordance.

The abundance of lymphoreticular cells in the liver and intestine, plus the development of phenotyping techniques,[8,14,22] have contributed to the discovery of cell migration and repopulation that follows organ transplantation. We believe that cell migration takes place to some degree with all successful transplantations, irrespective of the organ, with rapid seeding through the blood stream. As far back as 1962–63, we found evidence that cells migrated from kidney allografts into recipients treated with azathioprine and prednisone.[23] After renal transplantation, previously negative tuberculin, histoplasmin, and other skin tests among recipients always became positive to antigens that had been shown to provoke positive reactions in their donors. These results were interpreted as showing adoptive transfer of donor cellular immunity "by leukocytes in the renal vasculature and hilar lymphoid tissue".[24]

At the time, alteration of graft antigenicity was suggested as an explanation for the reversal of kidney rejection in these patients when prednisone was added to baseline therapy with azathioprine, and for the ability later to reduce maintenance immunosuppression.[23] More than 28 years later, chimerism as a reason for diminished antigenicity was demonstrated with immunocytochemical and PCR techniques in biopsy samples from the renal allograft, skin, and lymph nodes of several of these same patients (unpublished observations). Such reductions in antigenicity of free thyroid grafts, when located in the anterior chamber of the guinea pig eye before subcutaneous engraftment, had been observed by Woodruff and Woodruff[25] who called this process "adaptation".

We have also described cell repopulation of human heart-lung allografts from studies of necropsy samples of recipients treated with cyclosporin.[26] In untreated rats, Prop et al[27] showed that a lymphoid-poor heart is less vigorously rejected than a lung that contains rich bronchus-associated lymphoid tissue (BALT). However, this order of susceptibility to rejection was reversed with postoperative cyclosporin, which often induced permanent acceptance of the rat lung, but never of the heart. This paradox was explained by the greater volume and ease of migration of the lung's lymphoid and dendritic cell population. Permanent graft acceptance in rats after a brief induction course of FK 506 has also been shown to be more difficult to achieve with the heart than with the liver,[28] a difference that is reflected by the difficulty of maintenance of seeded peripheral donor cells after cardiac but not liver engraftment.[8]

Thus, cell traffic seems to be a striking event with all transplants. Donor cells leaving the solid organ graft and recipient cells entering it include passenger leucocytes that were shown by Steinmüller[29] to be the main cause of allograft immunogenicity. These cells are a distinct family of bone-marrow derived antigen presenting dendritic leucocytes.[30] These dendritic leucocytes are distributed throughout the body, including organs once thought to be mostly devoid of immunologically active cells.[30,31] The evidence implicating these antigen-presenting cells in primary T-cell alloimmunity[22,30,32] has prompted efforts to eliminate them before transplantation.[33,34]

Whether this reduction of graft antigenicity is beneficial remains uncertain. Our data show that migration of

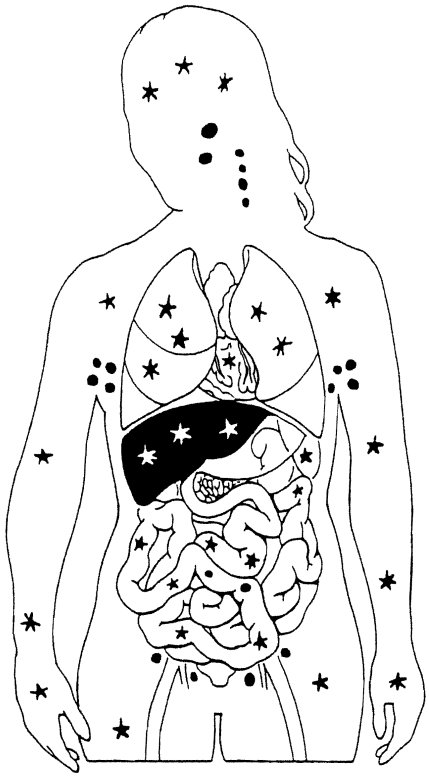

Fig 3—Result of traffic of donor and recipient lymphoreticular cell traffic after successful liver transplantation.

White, recipient cells; black, donor cells.

dendritic and lymphoid cells is associated with graft acceptance rather than rejection, depending on the quality of immunosuppression, the immunological substrate of the organs, donor-recipient histocompatibility, and perhaps other factors. The fine margin between graft rejection and acceptance was shown by Armstrong et al,[35] who found an association between the increased rate of dendritic cell replacement and the survival of renal allografts transplanted to rats after they had been immunised by blood transfusion from the donor strain.

Thus, contrary to the dendritic cell deletion approach, the objective perhaps should be to promote, not prevent, two-way cell migration while at the same time giving treatment to avoid graft destruction or GVHD, which are the normal and inevitable consequences of migration. If so, improved treatment strategies might include perioperative infusion of bone-marrow or other immunocompetent cells.[36-38] Antigen extracts or killed cells cannot substitute for living cells.[38,39]

We have not attempted to distinguish between drug-free "classical tolerance", as defined by Billingham, Brent, and Medawar,[36] the tolerance found after bone-marrow transplantation, and the ambiguous "graft acceptance" referred to by solid organ transplant surgeons. We believe that all are variants or stages of the same cell migration process. Clinical success—tolerance or graft acceptance—means that a characteristic lymphoid and dendritic cell chimerism has been introduced, which may be stable either without further treatment or only when continued immunosuppression is provided; an unstable graft and its migrated cells may either be rejected or cause GVHD. Thus, our view of solid organ graft acceptance can be related easily to the Billingham-Brent-Medawar model of actively

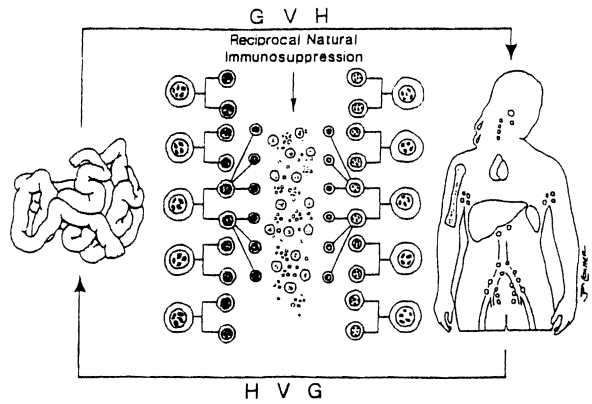

Fig 4—Reciprocal clonal expansion and depletion by immune cells in graft and recipient.

HVG: host *vs* graft.

acquired tolerance,[36,40] and accommodates Woodruff's suggestion about "replacement of certain elements of graft, for example connective tissue stroma and vascular endothelium".[41]

Medawar was perplexed by the unexpected success of clinical renal transplantation and wrote that ". . . foreign kidneys do sometimes become acceptable to their hosts for a reason other than acquired tolerance in a technical sense . . . One possible explanation is the progressive and perhaps very extensive replacement of the vascular endothelium of the graft by endothelium of host origin, a process that might occur insidiously and imperceptibly during a homograft reaction weakened by immunosuppressive drugs".[42] He was unaware of the existence of cell migration and its possible association with transplant tolerance.

Since cell migration quickly transforms both the graft and the recipient into chimeras, both the importance of HLA matching for bone-marrow transplantation (an inherently immunologically unbalanced procedure) and its imperfection as a predictor for outcome of solid organ transplantation[43,44] might now have an explanation. Although the dynamics of the chimeric state remain speculative, the reciprocal clonal deletion that was invoked to explain GVHD resistance (fig 4) can be thought of as immunosuppressive in proportion to the degree of MHC incompatibility. This process would be especially important with organs such as the liver and intestine, which have an important immunological component.

Cell migration, which we believe is an invariable early event in graft acceptance, could lead to self-perpetuating and presumably linked changes in the host immune response, which do not depend on the continued survival of seeded donor cells. Hypotheses such as these have defied attempts at verification,[45] probably because the proposed elements of each theory are simply epiphenomena of the key event: cell migration and repopulation.

This work was supported by Project Grant DK 29961 from the National Institutes of Health, Bethesda, Maryland.

REFERENCES

1. Kashiwagi N, Porter KA, Penn I, Brettschneider L, Starzl TE. Studies of homograft sex and of gamma globulin phenotypes after orthotopic homotransplantation of the human liver. *Surg Forum* 1969; 20: 374–76.
2. Porter KA. Pathology of the orthotopic homograft and heterograft. In: Starzl TE, ed. Experience in hepatic transplantation. Philadelphia: WB Saunders, 1969: 464–05.
3. Kashiwagi N. Special immunochemical studies. In: Starzl TE, ed.

Experience in hepatic transplantation. Philadelphia: WB Saunders, 1969: 394–407.
4. Ramsey G, Nusbacher J, Starzl TE, Lindsay GD. Isohemagglutinins of graft origin after ABO-unmatched liver transplantation. *N Engl J Med* 1984; 311: 1167–70.
5. Davies HFFS, Pollard SG, Calne RY. Soluble HLA antigens in the circulation of liver graft recipients. *Transplantation* 1989; 47: 524–27.
6. Singh PB, Brown RE, Roser B. Class I transplantation antigens in solution in body fluids and in the urine. *J Exp Med* 1988; 168: 195–211.
7. Selby R, Starzl TE, Yunis E, Brown BI, Kendall RS, Tzakis A. Liver transplantation for type IV glycogen storage disease. *N Engl J Med* 1991; 324: 39–42.
8. Demetris AJ, Murase N, Starzl TE. Donor dendritic cells after liver and heart allotransplantation under short-term immunosuppression. *Lancet* 1992; 339: 1610.
9. Ricordi C, Ildstad ST, Demetris AJ, Abou El-Ezz AY, Murase N, Starzl TE. Donor dendritic cell repopulation in recipients after rat-to-mouse bone-marrow transplantation. *Lancet* 1992; 339: 1610–11.
10. Starzl TE, ed. Experience in hepatic transplantation. Philadelphia: WB Saunders, 1969: 198–206, 226–33.
11. Starzl TE, Ishikawa M, Putnam CW, et al. Progress in and deterrents to orthotopic liver transplantation, with special reference to survival, resistance to hyperacute rejection, and biliary duct reconstruction. *Transplant Proc* 1974; 6: 129–39.
12. Calne RY, Sells RA, Pena JR, et al. Induction of immunological tolerance by porcine liver allografts. *Nature* 1969; 233: 472–76.
13. Murase N, Demetris AJ, Matsuzaki T, et al. Long survival in rats after multivisceral versus isolated small bowel allotransplantation under FK 506. *Surgery* 1991; 110: 87–98.
14. Iwaki Y, Starzl TE, Yagihashi A, et al. Replacement of donor lymphoid tissue in human small bowel transplants under FK 506 immunosuppression. *Lancet* 1991; 337: 818–19.
15. Arnaud-Battandier F, Salmon H, Vaiman M, et al. Small intestine allotransplantation in swine with cyclosporine treatment: studies of the intestinal lymphoid populations. *Transplant Proc* 1985; 17: 1440–41.
16. Murase N, Demetris AJ, Woo J, et al. Lymphocyte traffic and graft-versus-host disease after fully allogeneic small bowel transplantation. *Transplant Proc* 1991; 23: 3246–47.
17. Murase N, Demetris A, Wood J, et al. Graft-versus-host disease (GVHD) after BN to LEW compared to LEW to BN rat intestinal transplantation under FK 506. *Transplantation* (in press).
18. Starzl TE, Todo S, Tzakis A, et al. The many faces of multivisceral transplantation. *Surgery Gynecol Obstet* 1991; 172: 335–44.
19. Todo S, Tzakis A, Abu-Elmagd K, et al. Intestinal transplantation in composite visceral grafts or alone. *Ann Surg* (in press).
20. Ildstad ST, Sachs DH. Reconstitution with syngeneic plus allogeneic or xenogeneic bone marrow leads to specific acceptance of allografts or xenografts. *Nature* 1984; 307: 168.
21. Webb S, Morris C, Sprent J. Extrathymic tolerance of mature T cells: clonal elimination as a consequence of immunity. *Cell* 1990; 63: 1249–56.
22. Demetris AJ, Qian S, Sun H, et al. Early events in liver allograft rejection. Delineation of sites of simultaneous intragraft and recipient lymphoid tissue sensitization. *Am J Pathol* 1991; 138: 609–18.
23. Starzl TE, Marchioro TL, Waddell WR. The reversal of rejection in human renal homografts with subsequent development of homograft tolerance. *Surg Gynecol Obstet* 1963; 117: 385–95.
24. Wilson WEC, Kirkpatrick CH. Immunologic aspects of renal homotransplantation. In: Starzl TE, ed. Experience in renal transplantation. Philadelphia: WB Saunders, 1964: 239–61.
25. Woodruff MFA, Woodruff HG. The transplantation of normal tissues: with special reference to auto- and homotransplants of thyroid and spleen in the anterior chamber of the eye, and subcutaneously, in guinea pigs. *Phil Trans R Soc Lond (Biol)* 1950; 234: 559–81.
26. Fung JJ, Zeevi A, Kaufman C, et al. Interactions between bronchoalveolar lymphocytes and macrophages in heart-lung transplant recipients. *Hum Immunol* 1985; 14: 287–94.
27. Prop J, Kuijpers K, Petersen AH, Bartels HL, Nieuwenhuis P, Wildevuur CRH. Why are lung allografts more vigorously rejected than hearts? *Heart Transplantation* 1985; 4: 433–36.
28. Murase N, Kim DG, Todo S, Cramer DV, Fung JJ, Starzl TE. Suppression of allograft rejection with FK 506. I: prolonged cardiac and liver survival in rats following short course therapy. *Transplantation* 1990; 50: 186–89.
29. Steinmuller D. Immunization with skin isografts taken from tolerant mice. *Science* 1967; 158: 127–29.
30. Steinman RM. The dendritic cell system and its role in immunogenicity. *Annu Rev Immunol* 1991; 9: 271–96.
31. Hart DNJ, Fabre JW. Demonstration and characterization of Ia-positive dendritic cells in the interstitial connective tissues of rat heart and other tissues, but not brain. *J Exp Med* 1981; 154: 347–61.
32. Lechler RI, Batchelor JR. Restoration of immunogenicity to passenger cell-depleted kidney allografts by the addition of donor-strain dendritic cells. *J Exp Med* 1982; 155: 31.
33. Talmage DW, Dart G, Radovich J, Lafferty KJ. Activation of transplant immunity: effect of donor leukocytes on thyroid allograft rejection. *Science* 1976; 191: 385–87.
34. Faustman D, Hauptefeld V, Lacy P, Davie J. Prolongation of murine islet allograft survival by pretreatment of islets with antibody directed to Ia determinants. *Proc Natl Acad Sci USA* 1981; 78: 5156–59.
35. Armstrong HE, Bolton EM, McMillan I, Spencer SC, Bradley JA. Prolonged survival of actively enhanced rat renal allografts despite accelerated cellular infiltration and rapid induction of both class I and class II MHC antigens. *J Exp Med* 1987; 164: 891–907.
36. Billingham R, Brent L, Medawar P. Quantitative studies on tissue transplantation immunity. III. Actively acquired tolerance. *Phil Trans R Soc Lond (Biol)* 1956; 239: 357–412.
37. Slavin S, Strober S, Fuks Z, Kaplan HS. Induction of specific tissue transplantation tolerance using fractionated total lymphoid irradiation in adult mice: long-term survival of allogeneic bone marrow and skin grafts. *J Exp Med* 1977; 146: 34–48.
38. Monaco AP, Wood ML, Maki T, Gozzo JJ. Post-transplantation donor-specific bone marrow transfusion in polyclonal antilymphocyte serum-treated recipients: the optimal cellular antigen for induction of unresponsiveness to organ allografts. *Transplant Proc* 1988; 20: 1207–12.
39. Cranston D, Foster S, Wood KJ, Morris PJ. The combined effect of perioperative donor spleen cells or KCl-extracted antigen and cyclosporine on renal allograft survival in the rat. *Transplantation* 1991; 52: 789–94.
40. Billingham RE, Brent L, Medawar PB. "Actively acquired tolerance" of foreign cells. *Nature* 1953; 172: 603–06.
41. Woodruff MFA. Evidence of adaptation of homografts of normal tissue. In: Medawar PB, ed. Biological problems of grafting. Oxford: Blackwell, 1959: 83–94.
42. Medawar PB. Transplantation of tissues and organs: introduction. *Br Med Bull* 1965; 21: 97–99.
43. Gjertson D, Terasaki P, Takamoto S. National allocation of cadaveric kidneys by HLA matching: projected effect on outcome and costs. *N Engl J Med* 1991; 324: 1032.
44. Markus BH, Duquesnoy RJ, Gordon RD, et al. Histocompatibility and liver transplant outcome: does HLA exert a dualistic effect? *Transplantation* 1988; 46: 372–77.
45. Murase N, Kim DG, Todo S, Cramer DV, Fung JJ, Starzl TE. FK 506 suppression of heart and liver allograft rejection II: the induction of graft acceptance in rat. *Transplantation* 1990; 50: 739–44.

From The Lancet

Medicine and war

The health of armies and fleets has been for many years considered an object of the highest national importance. Commanders by land or sea, who have deserved to be called great, have always manifested the utmost solicitude on this point, and, however lavish of the blood of their followers on those days of struggle that decide the fate of nations, were most careful of their health and vigour, and so were enabled to take advantage of the moment, and to win their battles. It is said of Wellington that at the most critical juncture of his career his thoughts were chiefly occupied with his soldiers' shoes; and the heroic Nelson is known to have made it his boast that in a small vessel he commanded in his youth he had not lost a man by sickness. In the wars of the last century, when the military art became more highly organised than in the half-feudal times of the Tudors and Stuarts, great attention began to be paid to the health of troops and seamen by their medical officers. While Sir John Pringle and others were endeavouring to save armies in the field, Lind, Trotter, Blane, and other distinguished naval men made hygiene or preventive medicine their special study, and with more striking results. On board ship, sanitary regulations can be applied with greater precision and exactitude than anywhere else. Some most interesting experiments were made as to the influence of diet, climate, &c, both at home and abroad, and the naval medical officers of that time may be justly accounted the founders, in this country at least, of sanitary science. Their works are replete with instruction, and many useful hints may even now be gathered from them.

(March 2, 1867)