1604

Hematolymphoid Cell Trafficking, Microchimerism, and GVH Reactions After Liver, Bone Marrow, and Heart Transplantation

A.J. Demetris, N. Murase, S. Fujisaki, J.J. Fung, A.S. Rao, and T.E. Starzl

THE RECENT discovery of ubiquitously located small numbers of donor leukocytes (microchimerism) in human kidneys, livers, and other organs[1–6] up to 29 years posttransplantation has raised questions about the migration of the chimeric cells and their role in the induction and perpetuation of graft acceptance. In the human studies, the most prominent of the peripheralized donor leukocytes appeared morphologically to be the dendritic cells delineated as a special white cell lineage in 1973 by Steinman and Cohn[7–10] and normally associated with organ immunogenicity rather than tolerogenicity.[11–12] We report here an investigation of the cell migration in unmodified versus immunosuppressed Brown Norway (BN) rat recipients of Lewis (LEW) livers with emphasis on the kinetics, location, and the reaction elicited by these cells in the recipient lymphoid organs.

We also looked for histopathologic signs in these animals of the graft versus host (GVH) reaction that we have postulated to be an integral part of organ graft acceptance.[1,5,6] To magnify the GVH effect, we developed a model in which the lethal GVHD potential of the liver passenger leukocytes could be routinely demonstrated in the LEW to BN strain combination.

MATERIALS AND METHODS

Animals and Procedures

Male Lewis (LEW, $RT1^l$) and Brown Norway (BN, $RT1^n$) rats (250 to 300 g) (Harlan Sprague Dawley, Inc. Indianapolis, Ind) were used as donors and recipients, respectively. All procedures and killings were under methoxyflurane anesthesia. Orthotopic liver transplantation (liver replacement) was with the cuff technique of Kamada and Calne,[13] without arterial reconstruction. Heterotopic heart transplantation was to the abdominal location with anastomosis of the graft aorta and pulmonary artery to the recipient's infrarenal aorta and inferior vena cava, respectively.[14]

Bone marrow was taken from the tibias and femurs and processed in RPMI 1640 supplemented with 25 mmol/L HEPES buffer, 2 mmol/L L-glutamine, penicillin (50 U/mL) and streptomycin (50 μg/mL) (Gibco, Grand Island, NY).

Characterization of Cell Migration

Without Immunosuppression. Because fatal liver rejection with LEW → BN does not occur until 23 to 37 days (n = 10, see footnote Table 1, and group 2, Table 2), all animals (n = 12) were in good condition until sacrifice on days 3, 5, 7, and 14 (n = 3 each). A piece of tissue from the liver graft, and recipient spleen, thymus, heart, tongue, and cervical and mesenteric lymph nodes were fixed in formalin for routine histopathology and a separate portion frozen for immunohistochemical studies.

With Immunosuppression. Rats were treated with 1.0 mg/kg/d FK 506 for 14 days, and once a week thereafter except for two animals kept for 300 postoperative days after stopping treatment at 28 days. Three animals each were sacrificed on day 3, 5, 7, and 14 with further sacrifices on day 28 (n = 4), 100 (n = 2), and 300 (n = 3). Of the three rats maintained for 300 days, one received weekly FK 506 until the end (Table 1, group 11) while two were without treatment after the fourth postoperative week (Table 1, group 12). The tissue collections were the same as in the untreated rats (Table 1), and in addition bone marrow was examined from the rats kept for 3 and 300 days.

Recipient and Donor Cell Proliferative Response

In the cell migration experiments, the proliferative response in the spleen and other lymphoid organs was monitored with two methods. Metaphase mitotic figures were counted in a standardized area of tissue as previously reported.[15] In addition, each animal was injected 1 hour before sacrifice with 50 mg/kg IV of 5-bromo-2′-deoxyuridine (BrdU, Sigma Chemical Co., St. Louis, Mo) in preparation for double immunolabelling studies of proliferating cells.

Cell Phenotyping

The monoclonal antibody L-21-6, which reacts with the invariant chain of class II MHC antigens of LEW and most other rat strains but not BN,[16] was used to differentiate donor class II MHC positive cells from recipient cells in the tissues. For identification of donor (L-21-6+) cells, the previously described avidin-biotin peroxidase method for localizing the bound primary antibody[15] was used with one modification. H_2O_2/methanol quenching of endogenous peroxidase activity was delayed until after incubation with the primary antibody, because the antigen recognized was found to be H_2O_2/methanol sensitive (unpublished observation). Normal LEW and BN spleens were used in each run as positive and negative controls, respectively, and an irrelevant primary antibody was substituted on each section as an additional negative control. The number of donor cells in recipient tissue was semiquantitatively estimated by counting three separate high power fields (HPF; 400× magnification) in tissue sections of the organ examined.

For the studies of the identification of proliferating cells in the lymphoid organs, donor MHC class II+ cells were identified by the above technique, and then the dividing cells were localized

From the Pittsburgh Transplantation Institute, and the Departments of Pathology (A.J.D., A.S.R.) and Surgery (N.M., S.F., J.J.F., A.S.R., T.E.S.), University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania.

Aided by Project Grant No. DK 29961 from the National Institutes of Health, Bethesda, Maryland.

Address reprint requests to Thomas E. Starzl, MD, PhD, Department of Surgery, 3601 Fifth Avenue, 5C Falk Clinic, University of Pittsburgh, Pittsburgh, PA 15213.

0041-1345/93/$3.00/+0

Table 1. Number of L-21-6+ Cells Per High-Powered Field (HPF) in Recipient (BN) Tissues at Various Times After Liver Transplantation in Treated and Untreated Recipients*,†

Group	Days Post-Tx	Number Animals	Treatment	Spleen	MLN	CLN	Heart	Tongue
1	3	3	None	17	16	28	0	0
2	5	3	None	7	6	9	0	0
3	7	3	None	2	3	3	0	0
4	14	3	None	0	0	0	0	0
5	3	3	FK 506	16	17	11	0	0
6	5	3	FK 506	12	11	15	0	0
7	7	3	FK 506	5	6	7	0	0
8	14	3	FK 506	4	7	7	0	<1
9	28	4	FK 506	5	8	9	<1	<1
10	100	2	FK 506	4	14	14	<1	>1‡
11	300	1	FK 506§	<1	<1	<1	<1	<1
12	300	2	FK 506‖	0	<1	0	0	<1

*The survival of 10 untreated liver recipients (controls) was 23, 23, 23, 25, 28, 29, 29, 30, 32 and 37 days.
†The values shown represent the mean.
‡One had histologic evidence of low-grade GVHD.
§Treated once a week up to the time of sacrifice. Donor cells were 4–5× more plentiful than in the untreated animal (Group 12) at the same time of follow-up.
‖Untreated after 28 days.
Abbreviations: MLN, mesenteric lymph node; CLN, cervical lymph node; Tx, transplantation.

with the monoclonal antibody against the incorporated 5-bromo-2′-deoxyuridine (BrdU; Amersham International plc; Amersham, UK).

Attempts to Categorize Lineages

Efforts with double immunolabelling to distinguish tissue macrophages, dendritic cells, and T and B cells were frustrated by the weak and often ambiguous staining obtained when directly labelled (FITC conjugated), commercial reagents were used as the second primary immunoreactants.

Experiments Augmenting GVH

BN rats were infused with 2.5×10^8 LEW or BN (control) bone marrow cells via the penile vein on day 0 and treated then and for 13 more days with 1.0 mg/kg/d FK 506, followed by single drug doses on days 20 and 27. All immunosuppression was then stopped. LEW liver (group 12, Table 2) or heart transplantation

Table 2. IV Cell Augmentation With 2.5×10^6 Bone Marrow (BM) Cells in FK 506-Treated BN Rats That Were Contemporaneously or Subsequently Given a Heart or Liver Allograft

Group	Bone Marrow (source)	FK 506†	LEW Organ Tx	Days Tx‡ After FK 506	Chimerism	Rejection	Survival After Tx
Untreated Whole Organ Tx							
1	None	No	Heart	NA	NT	+++	7, 8, 8, 8, 8, 8, 10, 11
2	None	No	Liver	NA	No	+++	23, 23, 23, 25, 28, 29, 29, 30, 32, 37
FK 506: Whole Organ Plus Recipient BMTx (Syngeneic Controls)							
3	Yes (BN)	Yes	Heart	0§	NT	+++	102, 102, 102, 138
4	Yes (BN)	Yes	Heart	45‖	NT	+++	17, 17, 23
5	Yes (BN)	Yes	Liver	0§	Yes	0	104, 104, 106, >154, >154, >164
6	Yes (BN)	Yes	Liver	45‖	NT	0	132, 132, 132
FK 506: Whole Organ Plus Donor (Allogeneic) BMTx							
7	Yes (LEW)	No	No	NA	No	NA	91, 91
8	Yes (LEW)	Yes	No	NA	Yes	NA	30, 30, 100, 100, 100
9	Yes (LEW)	Yes	Heart	0§	Yes	+	100, 100, 100
10	Yes (LEW)	Yes	Heart	45‖	Yes	+	96, 96, 101, 102
11	Yes (LEW)	Yes	Liver	0§	Yes	+	54¶, 100
12	Yes (LEW)	Yes	Liver	45‖	Yes	0	21*, 22*, 22*, 23*, 37*

Note: Numbers underlined represent animals sacrificed for immunohistochemical studies.
*Recipients died from GVHD.
†1.0 mg/kg/d on day of BM injection (day 0) and for 13 days thereafter, and then on days 20 and 27.
‡The whole organ transplant times given are days that have lapsed from the beginning (day 0) of the 28 day course of FK 506.
§Bone marrow given and FK 506 started same day as organ transplantation.
‖Organ transplantation was 18 days after the last dose of the 28 day course of FK 506 and 45 days after the BM.
¶The death at 54 days was from a technical complication (biliary cirrhosis/cholangitis).
Abbreviations: NA, not applicable; Tx, transplantation; NT, not tested; BM, bone marrow.

(group 10, Table 2) was performed 45 days later, 18 days after the FK 506 treatment had ended. Histopathologic evidence of GVHD, chimerism, and rejection and the impact of these findings on mortality were the experimental end points.

In control experiments, liver or heart transplantation was performed on the same day as the bone marrow infusion rather than at a second stage (groups 9 and 11, Table 2). Other appropriate controls are given in Table 2.

RESULTS

Leukocytes in the Liver Allografts

Untreated Rats. As previously reported,[15] cell populations of the liver that normally do not express class II MHC antigens and therefore do not stain with L-21-6 antibody became universally (bile duct and sinusoid cells) or partly (hepatocytes) positive by day 5. This state persisted until the grafts failed between 23 and 37 days. Such changes in class II expression as a manifestation of rejection are well known.[17–20]

In contrast, nonparenchymal dendritic-shaped cells in the portal triads and in the perivenular and capsular connective tissue that normally express class II MHC rapidly decreased and were no longer detectable by day 5. The increasingly severe portal inflammatory cells signifying a fatal rejection were always L-21-6 negative, serving as an additional negative internal control for L-21-6 specificity.

Treated Rats. The changes in the control animals were almost completely prevented by immunosuppression. There was a mild mononuclear portal infiltrate on days 5 through 7 that was not seen in specimens obtained later except to a similarly minor degree at day 300 in the clinically well animals left untreated after 28 days. Biliary duct cells, sinusoidal cells, and hepatocytes were L-21-6− throughout except in two animals untreated after 28 days whose L-21-6+ bile ducts had focal epithelial damage suggestive of low grade rejection.

The nonparenchymal L-21-6+ (donor) cells that are normally found in the portal, perivenular, and capsular tissues remained plentiful for the first 100 days, but had been depleted to about 10% of normal in the animal treated continuously for 300 days. Cells in these locations that were dendritic or spindle-shaped appeared from phenotyping studies at 100 and 300 days to be of mixed recipient (L-21-6−) and donor (L-21-6+) origin.

Donor Chimeric Leukocytes in Recipient Tissues

Untreated Animals. The number of L-21-6+ (donor) cells in the recipient lymphoid organs was similar with or without immunosuppression for up to 5 days after transplantation. By 7 days, the donor class II-positive cells had diminished in the untreated animals, and by 14 days they were no longer detectable (Table 1). By this time, L-21-6 positivity was maximal in the rejecting liver.

Treated Animals. In contrast, all of the treated rats maintained their chimerism until the time of sacrifice. In the spleen at 3 days, donor (L-21-6+) cells were concentrated at the periphery of periarterial lymphatic sheath (PALS) B-cell follicles (Fig 1). Fewer round and dendritic-shaped donor cells were found at the interface between the T-cell dependent PALS and red pulp. At 14 and 30 days in the treated animals, donor cells were present in the inner PALS. At 100 days, donor cells were found in the marginal zone, follicles and rarely in the red pulp, and in the inner PALS (Fig 2). At 300 days, donor cells were less numerous but in the same locations as at 100 days.

The greatest concentration of L-21-6+ (donor) cells in the cervical and mesenteric lymph nodes at 3 days was in the cortical follicles, with the appearance of lymphocytes and cells with dendritic processes. There were fewer such cells in the interfollicular cortex. The donor cells in the paracortex were small and blastic. With the passage of time, the donor cells became more diffusely distributed throughout the lymph node cortex and paracortex. At 100 days and thereafter, small round L-21-6+ cells resembling B lymphocytes were found in primary cortical follicles. Cells that looked like macrophages and dendritic cells were found in the paracortex. The number of L-21-6+ cells at 300 days was much less than at 100 days.

In the thymus, the evolution was much the same as in the other lymphoid organs with the appearance in 3 to 5 days of L-21-6+ cells in the medullary parenchyma as well as round cells in medullary septal B cell follicles (Fig 3). Rare round or dendritic shaped cells could be seen in the shrunken medulla at 28 days, often at the corticomedullary junction, and rare donor cells still could be found in the periadventitia of the medullary vessels out to 100 days. By this time, the medulla was severely atrophic or absent, and at 300 days a normal thymic medullary architecture could no longer be recognized.

The bone marrow was examined in several animals at day 3, and in two of the three survivors at 300 days (one on and the other off weekly FK 506 treatment). Strongly and weakly positive L-21-6 round cells were present at 3 days and numbered about 1/5 HPF. Small round weakly L-21-6+ donor cells were found in the marrow of both long-term survivors. The donor cells averaged <1/30 HPF, with more in the animal *off* immunosuppression for 9 months, than in the chronically treated rat.

Beginning at 2 to 4 weeks, smaller numbers of donor class II-positive cells appeared in the tongue (or skin) and heart (Table 1). In both locations, these cells most commonly had spindle and dendritic shapes. In the tongue (Fig 4), they were located between dermal collagen bundles, in the periadventitia of deep dermal arteries or surrounding small superficial dermal capillaries, or in the perineural space. In one rat, L-21-6+ donor cells were found at the dermal-epidermal junction of the skin at 100 days, when a low-grade GVHD was diagnosed histopathologically. In the three animals followed for 300 days, the rat under continuous therapy had approximately five times the number of extra lymphoid L-21-6+ cells as the two animals whose grafts had histopathologic evidence of low-grade rejection 270 days after stopping FK 506.

Lymphoid Proliferative Response to Liver Transplantation

A vigorous splenic proliferative response in untreated animals was muted by FK 506 treatment (Fig 5). In the treated recipients, host splenocyte proliferation peaked at 5 days, decreased toward baseline thereafter, but remained higher than that previously reported in normal BN rats or historical untreated BN-BN isograft controls.[15]

Double immunolabelling with L-21-6 and anti-BrdU showed that at 3 days (both treated and untreated animals), the L-21-6− proliferating recipient lymphoid cells formed clusters at the PALS periphery and in the red pulp. The red pulp clusters were not associated with L-21-6+ donor cells and were noticeably diminished in the FK 506 treated recipients. In contrast, the clusters at the PALS periphery were associated with L-21-6+ donor cells and not diminished by FK 506 therapy. BrdU nuclear labeling was also detected in 10% to 15% of L-21-6+ cells in treated and untreated animals (Fig 6). It could not be ruled out from microscopy that these were proliferating recipient T-cells surrounded by donor dendritic cell processes rather than being dividing donor cells.

Augmentation of Passenger Leukocyte GVH Reaction

Bone Marrow Alone. Long-term chimerism was not found after bone marrow transplantation in untreated rats (group 7, Table 2). When a 4-week induction course of FK 506 was used, chimerism was always present at 30 days and beyond without grossly detectable GVHD then or subsequently (group 8, Table 2). The density, but not the quality (data not shown) of this chimerism was similar to that following liver transplantation under comparable treatment conditions (compare with group 9, Table 1).

Bone Marrow Plus Simultaneous Liver Transplantation

Overt GVHD was not caused in two long-surviving animals submitted to contemporaneous LEW bone marrow and liver transplantation under FK 506 (group 11, Table 2), or in BN recipients of syngeneic bone marrow and LEW liver grafts treated with the same immunosuppression (groups 5 and 6, Table 2).

Bone Marrow Plus Staged Liver Transplantation. In contrast, when liver transplantation was performed 45 days after LEW donor bone marrow infusion (18 days after completion of the FK 506 course), all 5 animals died of severe GVHD 21 to 37 days later (group 12, Table 2).

Bone Marrow Plus Heart Transplantation. When the LEW heart, which was rejected by unmodified BN recipient in 11 days (group 1, Table 2), was transplanted as the test organ following bone marrow engraftment, cardiac survival greater than 100 days was achieved with no clinically detectable GVHD whether the transplantation was simultaneous with the bone marrow (group 9, Table 2) or 45 days later—18 days after completion of the FK 506 course (group 10, Table 2). In control animals who received syngeneic bone marrow under FK 506 for 4 weeks, LEW cardiac grafts given 45 days later, experienced a slightly prolonged survival (17 to 23 days), but were ultimately rejected (group 4, Table 2).

Histopathologic Studies. The long-surviving LEW liver grafts supplemented with simultaneous LEW bone marrow had no evidence of rejection. However, two of three of the LEW heart grafts transplanted simultaneously with LEW bone marrow had evidence of chronic rejection with obliterative arteriopathy, upregulation of class II MHC on the arterial endothelium, and a low-grade pericardial and interstitial mononuclear infiltrate. Much less severe changes were found in the recipients in whom the heart was transplanted at a second stage. Immunohistochemical studies of extrahepatic tissues of both the liver and heart recipients revealed typically distributed chimeric cells.

DISCUSSION

The early events of cell migration after allotransplantation have received scant attention.[21–24] The results described herein suggested by morphologic criteria that multiple leukocyte lineages are involved in addition to dendritic cells as we proposed earlier.[1–6] However, double immunolabelling did not allow precise identification of the lineages. Despite this limitation, an overview of the leukocyte traffic was obtained. During the first 3 to 5 postoperative days, donor cells homed to the lymph nodes, spleen, and thymus. The migratory pattern was not at first obviously affected by immunosuppression. Without treatment, the donor cells disappeared within a further few days, whereas the expected outcome with a short course of FK 506 was permanent low-level chimerism and liver graft survival.

After 2 weeks in the immunosuppressed animals, increasingly dispersed donor cells could be the product of pluripotent stem cells like those recently cultured by Inaba et al from mouse blood and bone marrow,[25] or alternatively, a population of more mature migratory leukocytes that had not reached terminal differentiation. The arrival of donor cells in the skin and heart after 2 to 4 weeks is not unique, since this occurs after bone marrow transplantation,[26] which liver transplantation resembles,[3] and also after allogeneic fetal liver transplantation.[27]

One action of drugs of the cyclosporine class includes, but is not limited to, selective inhibition of MHC-restricted alloantigen presentation.[28,29] Because FK 506 appeared in our experiments to deter the development of lymphoid cell proliferation spatially unassociated with donor class II MHC positive cells, it was suspected to have a similar action. However, such characterization of the immunosuppressive effects of drugs in terms of their site of disruption of the alloactivated T cell response cannot explain the donor specific nonreactivity and permanent acceptance of organ grafts that have been reported in animals after a short treatment course of every genuinely potent immunosuppressant during the last 30 years. We have postulated that the development of this nonresponsiveness requires bidirectional alloactivation of GVH as

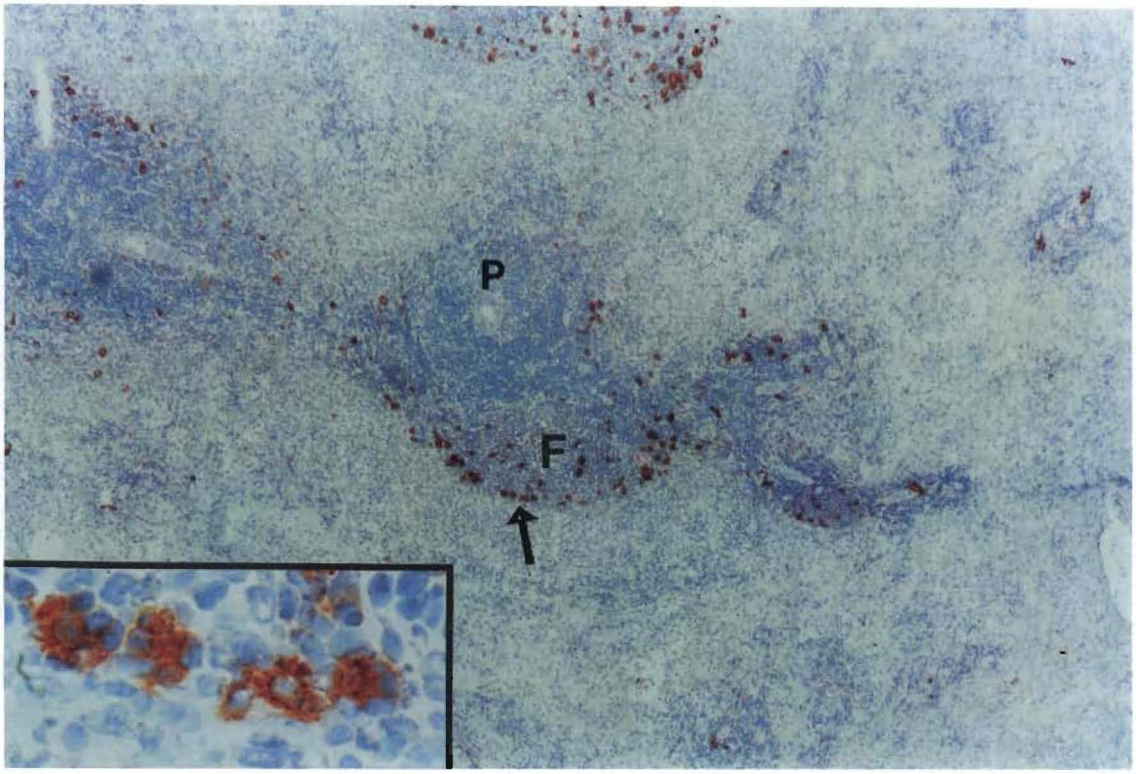

Fig 1. Recipient spleen 3 days after liver transplantation in rat treated with FK 506. MHC class II+ donor cells are lodged primarily in the periphery of periarterial lymphatic sheath (**P**) and B-cell follicles (**F**) (L-21-6 IPEX [red color] with hematoxylin counterstain; original magnification 40×). The inset (original magnification 400×) shows the staining detail of the donor cells lodged at the periphery of the follicles (small arrow).

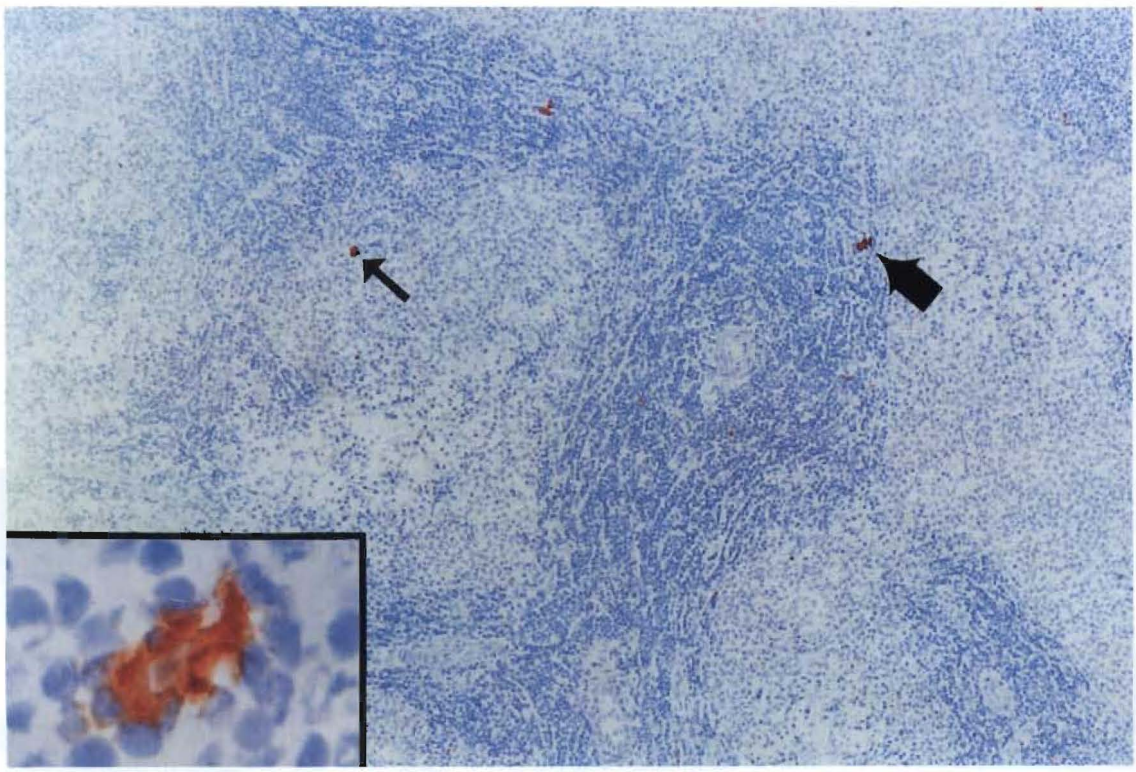

Fig 2. By 100 days posttransplant, donor MHC class II positive cells were less numerous in the spleen, but still easily detectable (L-21-6 IPEX [red color] with hematoxylin counterstain; original magnification 165×). A dendritic-shaped cell is seen in the PALS (large arrow) and a round cell in the follicle (small arrow). The inset (original magnification 330×) shows in greater detail the dentritic-shaped cell.

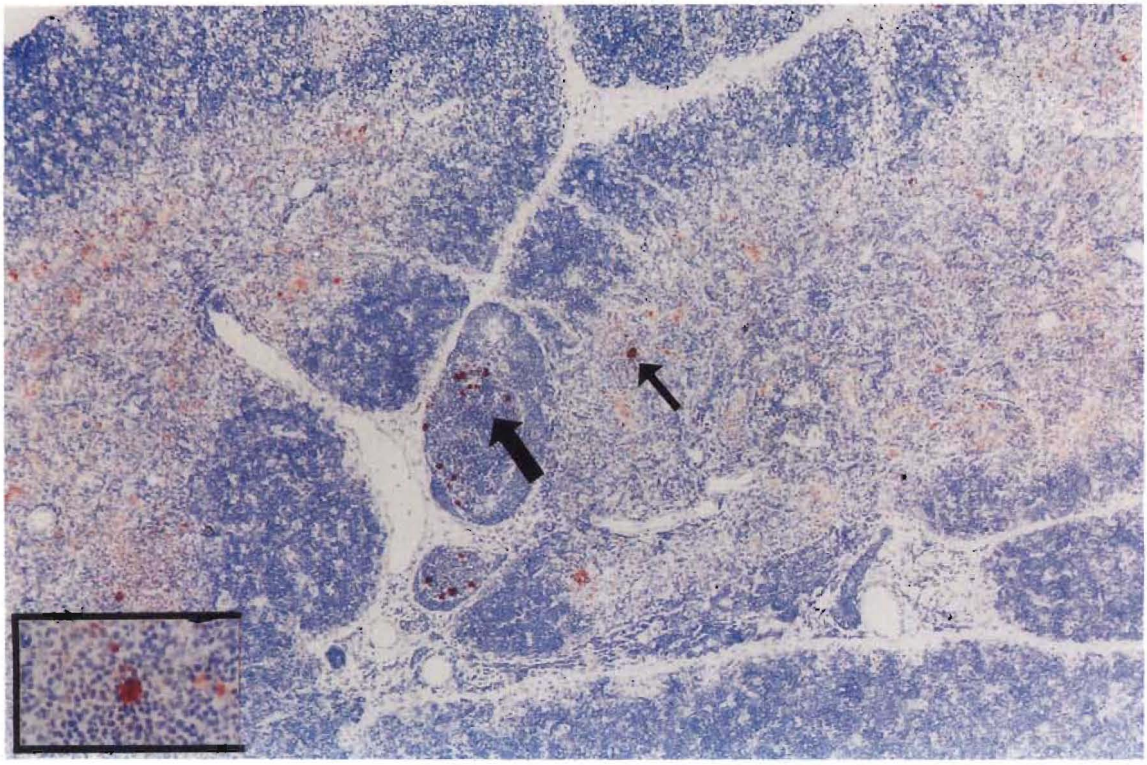

Fig 3. Donor MHC class II+ cells also migrated to the recipient thymus as early as 3 days after transplantation, but were few in number (L-21-6 IPEX) [red color] with hematoxylin counterstain; original magnification 40×). Note the 8 to 10 donor cells present in the small B-cell follicle (large arrow) and an isolated, similarly staining cell in the thymic medulla (small arrow). The inset shows the latter cell in greater detail (original magnification 100×).

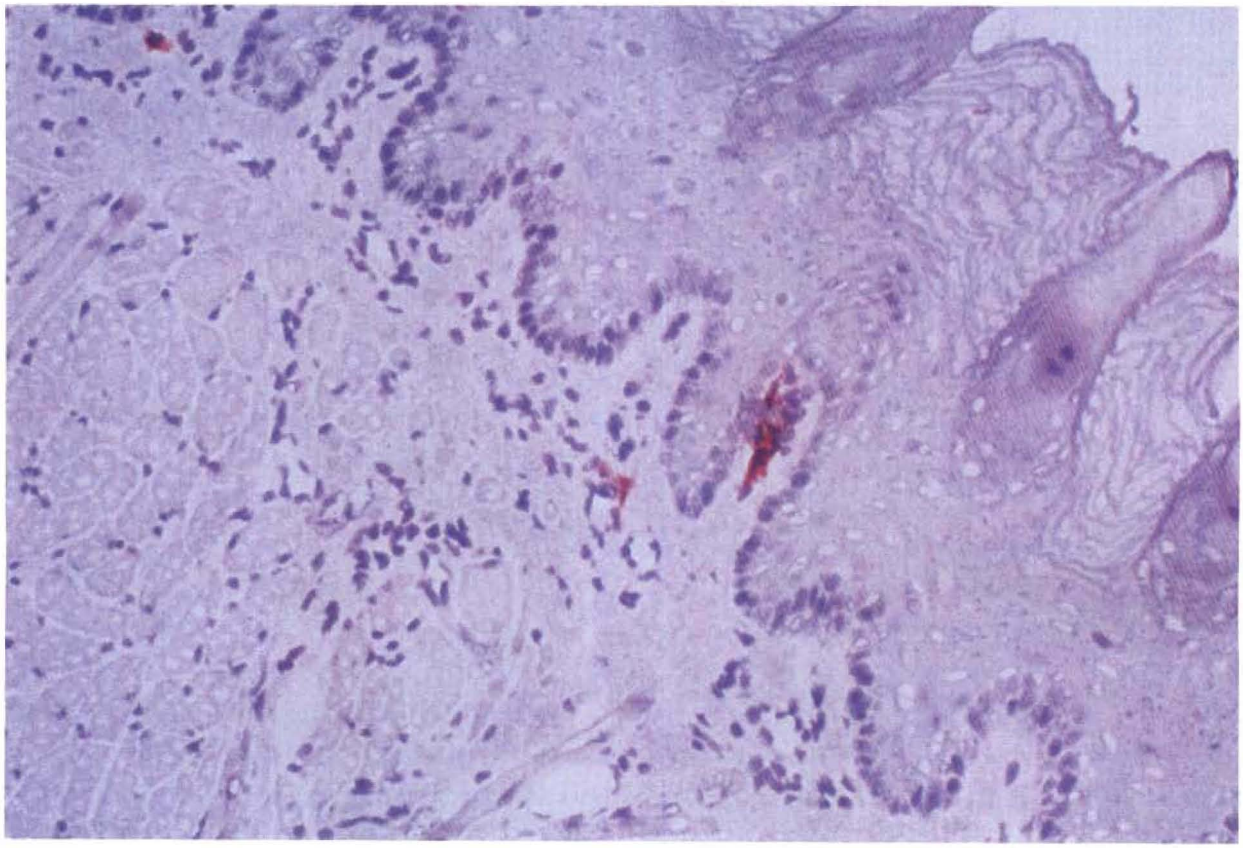

Fig 4. Donor MHC class II+ cells began to appear in the tongue as early as 30 days posttransplant, and were easily detectable at 100 days (L-21-6 IPEX [red color] with hematoxylin counterstain; original magnification 100×).

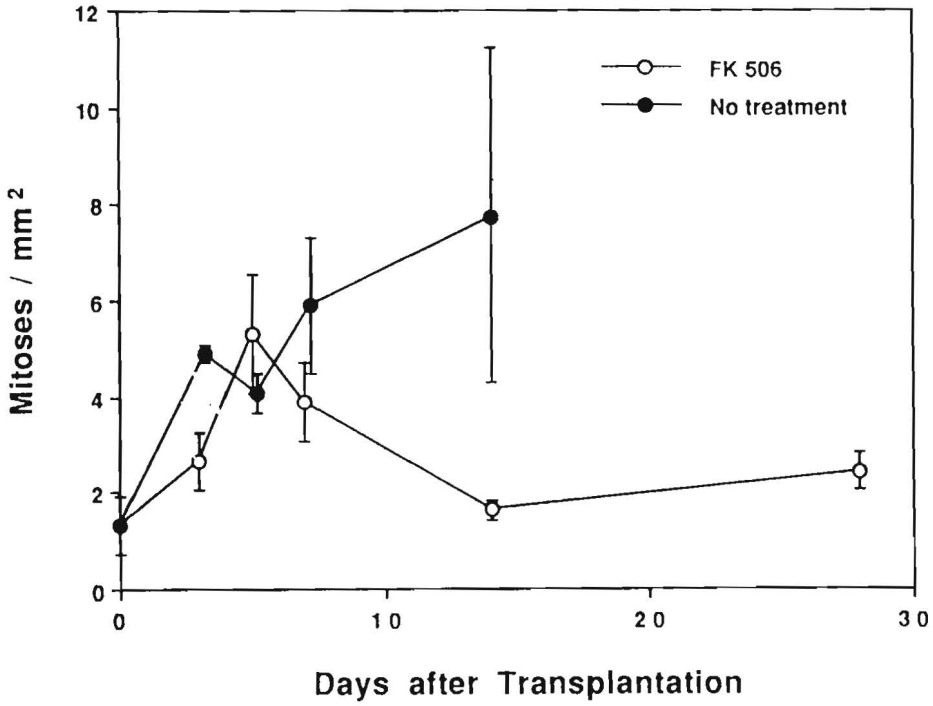

Fig. 5 Metaphase mitotic figure count/mm^2 in the recipient spleen after liver transplantation in FK 506-treated recipients (open circles) and untreated controls (closed circles). Note that FK 506 diminished but did not abolish the splenocyte proliferation.

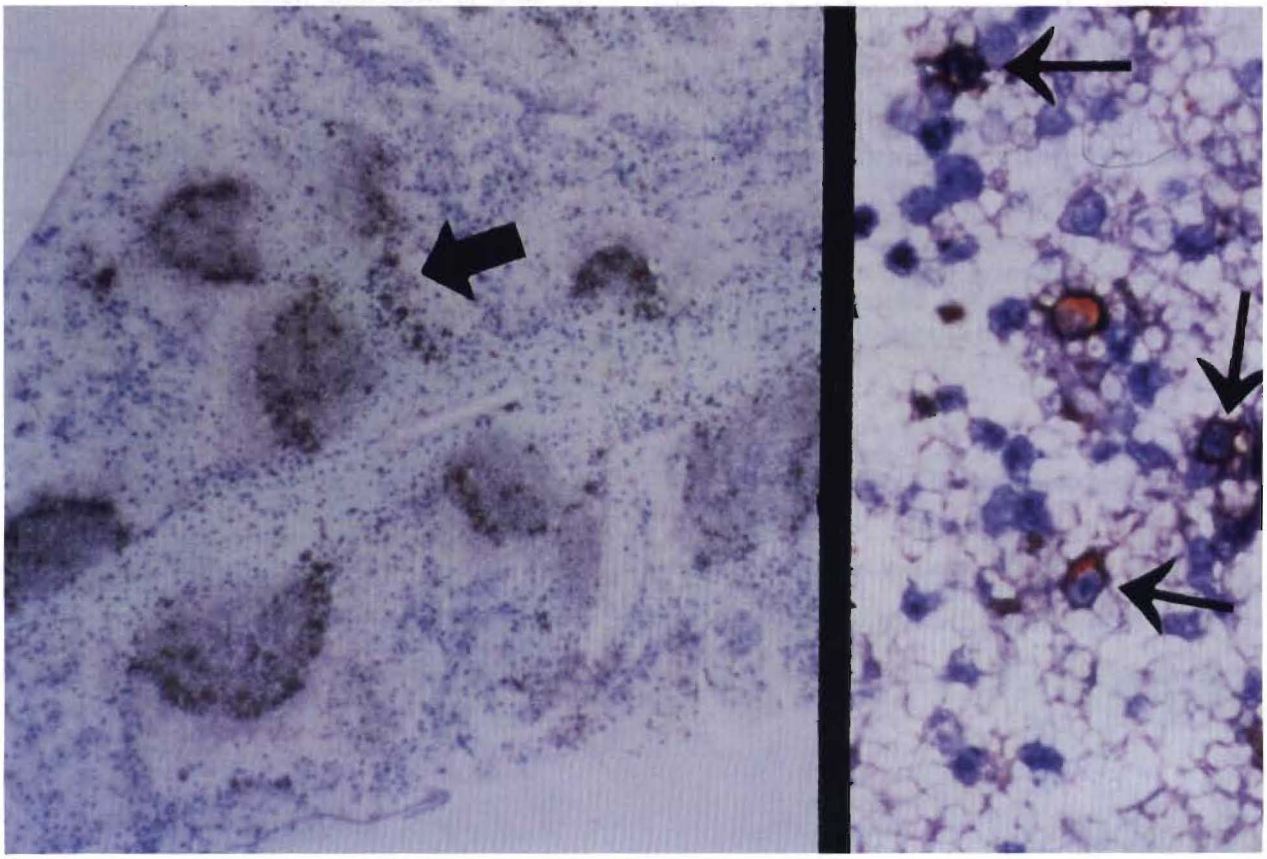

Fig 6. Recipient spleen 3 days after liver transplantation in a rat treated with FK 506. The tissue section was double labelled with L-21-6 (red) and proliferating cells (BrdU; blue nuclei). The left panel (original magnification 40×) shows MHC class II+ donor cells in the B-cell follicles and PALS (bold arrow). The PALS are shown at higher magnification in the right panel (original magnification 1000×). The cells with a blue nucleus and red cytoplasm (arrows) are thought to represent proliferating donor cells.

well host versus graft (HVG) varieties[6] whereby a portion of the donor immune system in a state of initially high- and then low-grade stimulation is incorporated into the existing and similarly activated host network.[30] Incompleteness of this assimilation is diagnosed by evidence of rejection.

How potentially powerful and reproducible the converse (GVH) reaction can be was unmasked by the staged experiments of liver transplantation plus donor strain bone marrow. These two procedures done simultaneously under immunosuppression did not cause GVHD. However, when chimerism was produced with preliminary bone marrow transplantation under FK 506, subsequent liver transplantation from the donor strain following a drug-free interval of 18 days invariably caused lethal GVHD, resembling the outcome of a parent-to-offspring F_1 hybrid experiment.[31] Under these circumstances, the liver including its virgin migratory cells was seen as self by the altered host immune system, but not having gone through the process of modification, the hepatic passenger leukocytes reciprocated by rejecting the recipient.

In contrast, heterotopic hearts transplanted under the same circumstances of prior bone marrow preparation were accepted after the second stage operation without causing GVHD. Presumably, this reflected the smaller load of heart passenger leukocytes. However, a contributing factor that cannot be arbitrarily dismissed is that the hearts were functionless auxiliary grafts whereas the livers not only replaced a significant part of the recipient immune apparatus but filled the void of parenchymal function left by host hepatectomy.

Further speculation about the way in which the microchimerism accompanying organ transplantation affects global recipient immunologic reactivity awaits delineation of the participating cells. This information in the mouse liver transplantation model is reported elsewhere.[32]

ACKNOWLEDGMENTS

We would like to thank Terry Mangan, Mary Ann Mient, and Pamela Slivinske for their editorial assistance. The technical assistance of Ms Beverly Gambrell is also acknowledged.

REFERENCES

1. Starzl TE, Demetris AJ, Murase N, et al: Lancet 339:1579, 1992

2. Starzl TE, Demetris AJ, Trucco M, et al: Lancet 340:876, 1992

3. Starzl TE, Demetris AJ, Trucco M, et al: New Engl J Med 328:745, 1992

4. Starzl TE, Demetris AJ, Trucco M, et al: Transplantation 55:1272, 1993

5. Starzl TE, Demetris AJ, Trucco M, et al: Hepatology 17:1127, 1993

6. Starzl TE, Demetris AJ, Murase N, et al: Immunol Today 14:326, 1993

7. Steinman RM, Cohn ZA: J Exp Med 137:1142, 1973

8. Steinman RM, Cohn ZA: J Exp Med 139:380, 1974

9. Steinman RM, Lustig DS, Cohn ZA: J Exp Med 139:1431, 1974

10. Steinman RM: Ann Rev Immunol 9:271, 1991

11. Hart DNJ, Winearls CG, Fabre JW: Transplantation 30:73, 1980

12. Lechler RI, Batchelor JR: J Exp Med 155:31, 1982

13. Kamada N, Calne RL: Transplantation 28:47, 1979

14. Ono K, Lindsey ES: J Thorac Cardiovasc Surg 7:225, 1969

15. Demetris AJ, Qian S, Sun H, et al: Am J Pathol 138:609, 1991

16. Murase N, Demetris AJ, Matsuzaki T, et al: Surgery 110:87, 1991

17. Takacs L, Szende B, Monostori E, et al: Lancet 2:1500, 1983

18. Demetris AJ, Lasky S, Van Thiel DH, et al: Transplantation 40:504, 1985

19. Gouw AS, Houthoff HJ, Huitema S, et al: Transplantation 43:291, 1987

20. Steinhoff G, Wonigeit K, Pichlmayr R: Transplantation 45:394, 1988

21. Murase N, Demetris AJ, Woo J, et al: Transplantation 55:1, 1993

22. van Schilfgaarde R, Hermans P, van Breda Vriesman PJC: Transplant Proc 11:1485, 1979

23. van Schilfgaarde R, Hermans P, Terpstra JL, et al: Transplantation 29:209, 1989

24. Murase N, Demetris AJ, Woo J, et al: Transplant Proc 23:3246, 1991

25. Inaba K, Inaba M, Romani N, et al: J Exp Med 176:1693, 1992

26. Good RA, Bach FH (eds): In: Clinical Immunobiology, Vol 2. New York: Academic Press, 1974, p 63

27. Huppes W, De Groot CJA, Ostendorf RH, et al: J Neurocytol 21:129, 1992

28. Kroczek RA, Black CDV, Barbet J, Shevach EM: J Immunol 139:3597, 1987

29. Clerici M, Shearer GM: J Immunol 144:2480, 1990

30. Demetris AJ, Murase N, Rao AS, Starzl TE: Twenty-fifth International Conference on Transplantation and Clinical Immunology, Lyon, France, 1993

31. Monchik GJ, Russell PS: Surgery 70:693, 1971

32. Qian S, Demetris AJ, Murase N, et al: Hepatology (in press)