Donor dendritic cells after liver and heart allotransplantation under short-term immunosuppression

SIR,—Donor (LEW) dendritic cell dissemination into recipient (BN) tissues was assessed in rats 16, 30, and 70 days after orthotopic liver transplantation (n = 3) and 30 days after intra-abdominal heart transplantation (n = 4), according to previously described surgical techniques.[1] Intramuscular FK 506 (1 mg/kg) was given to liver recipients daily for 7 days, but continued for 14 days in heart recipients because of the greater vulnerability to rejection of cardiac compared with hepatic grafts.[2] The same doses were then continued twice a week. Immunolabelling with a mouse monoclonal antibody, L-21-6, which recognises the invariant chain of LEW and most other rat strains, but not BN class II MHC antigen,[3] allowed the distinction of donor from recipient cells.

L-21-6+ (donor) dendritic-shaped cells were detected in lymphoid tissues of the non-rejecting LEW-BN liver recipients at 16, 30, and 70 days. These cells were restricted to the periarterial lymphatic sheaths of the spleen, and the paracortex of mesenteric, cervical, and intrathoracic lymph nodes, but diminished with time (figure). Occasional cells were also detected in the thymic medulla and non-lymphoid organs, such as the heart and skin, but not the brain. Liver allografts showed mild mononuclear infiltration with increased numbers of mast cells, but there was no evidence of parenchymal damage or obliterative arteriopathy. Residual donor (L-21-6+) dendritic cells were present in portal tracts and beneath the terminal hepatic venules at all time points examined.

Despite a longer period of daily FK 506 treatment, heart allografts had obliterative arteriopathy, indicating low-grade graft rejection. As in the liver transplants, the mild cellular infiltrate in heart allografts contained increased numbers of mast cells. An estimated one quarter of the normal quantity of dendritic cells in the cardiac graft interstitium and around blood vessels was identified as the persistent L-21-6+ (donor) phenotype. Unlike liver recipients, heart recipients had only rare donor dendritic cells in their spleen and lymph nodes, none in thymus, and none in non-lymphoid tissues, skin, lung, native heart, adrenal glands, kidneys, liver, intestines, and brain.

When liver transplantation was done across the immunologically more difficult ACI-BN strain barrier (n = 3), donor cell dissemination to extrahepatic tissues at 30 days resembled that after LEW-BN cardiac allotransplantation. Donor dendritic cells persisted irregularly in the liver grafts but were sparse or undetectable at extrahepatic sites.

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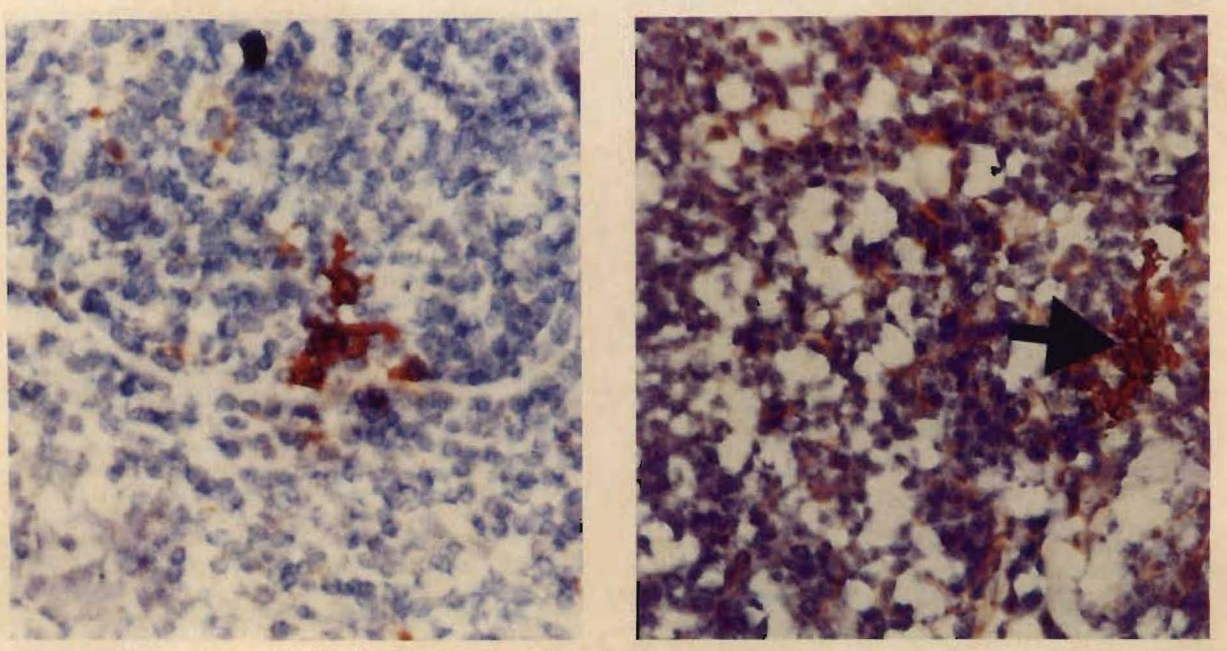
Donor dendritic cell repopulation in recipients after rat-to-mouse bone-marrow transplantation

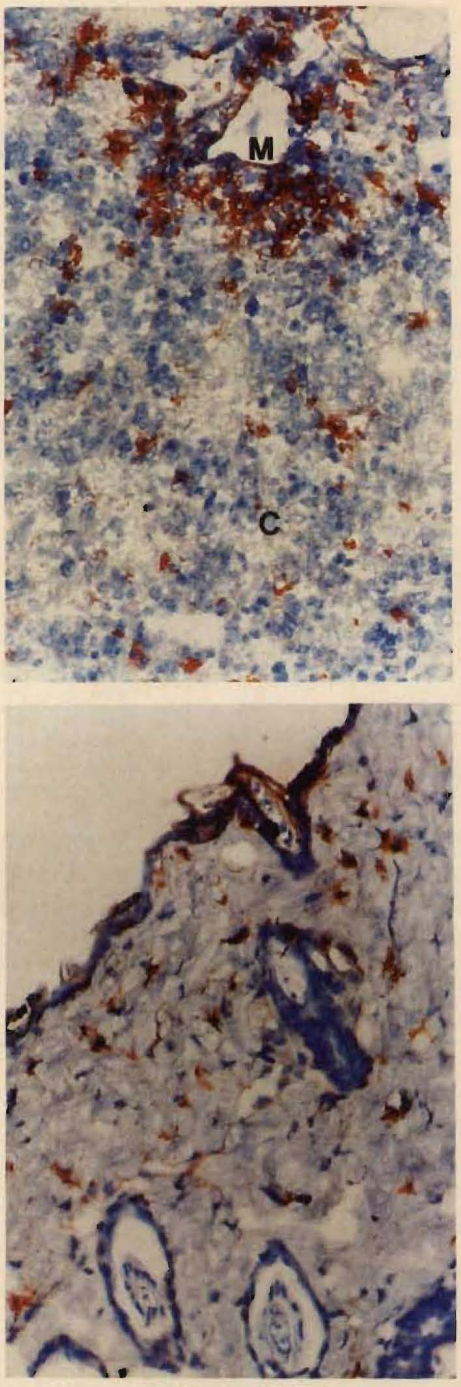
SIR,—Bone-marrow-derived dendritic cells are powerful antigen-presenting cells, which are 100-times more effective than macrophages in activating T lymphocytes in mixed-lymphocyte culture[1] and in intact animals.[2] These cells are pivotal in the generation of the T-cell repertoire, including provision of the appropriate ligand for negative selection of potentially autoreactive T lymphocytes.[3] We now describe the tissue distribution of donor dendritic accessory cells in fully xenogenic (F344 rat → B10 mouse) radiation bone-marrow chimeras that were permanently tolerant to donor-specific xenoantigens, yet fully reactive to third-party mouse and rat lymphoid cells.[4,5]

Laboratory animals were typed for chimerism by flow cytometry, and 3 per group were killed for a complete tissue survey at 1, 2, 3, 4, 6, and 8 weeks and at 8 months after reconstitution. Immunohistochemical staining for rat-derived dendritic cells (class II bright) was with the monoclonal antibody L-21-6 directed at the invariant chain of the rat class II molecule without cross-reactivity in mouse cells (provided by Dr Yuichi Iwaki, University of Pittsburgh).



Donor-recipient histological changes one month after rat liver allotransplantation under FK 506 immunosuppression.

Periarterial lymphatic sheath of spleen (left); thymic medulla (right). Immunoperoxidase stain with L-21-6 monoclonal antibody and haematoxylin counter stain (× 300– × 500).



Mouse thymus (top) and skin (bottom) six months after rat-to-mouse bone-marrow transplantation.

Top, staining by anti-MHC class II (L-21-6). Rust-coloured rat-derived cells are seen in both thymic cortex (C) and medulla (M) (× 300).

Bottom, L-21-6-positive-dendritic cells are shown in skin from the same animal (× 300).

In the first 2–3 weeks after reconstitution, the structure of the class-II-positive rat cells migrating into the tissue of the mice appeared more rounded than stellate and were thought to be an immature, less differentiated form of dendritic cell. By the end of one month and invariably thereafter, the rat dendritic cells were found in all of the mouse tissues examined: thymic cortex and medulla (figure, top), spleen, other lymphoid structures, liver, fat, peripheral nerve roots, brain (cerebellum, cerebrum), trachea, oesophagus, pancreas, lungs, bronchi, heart, and skin (figure, bottom). Their morphology, cell density, and distribution in the chimeras resembled that of normal rat tissues examined as controls.

The extent as well as the normal pattern of "homing" of the rat bone-marrow-derived dendritic cells in a xenogeneic mouse environment, suggests the presence of a highly conserved receptor-ligand mechanism. We are currently investigating the possible role of these ubiquitous cells in the induction and maintenance of specific transplantation tolerance across both allogeneic and xenogeneic histocompatibility barriers.

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Antimyeloperoxidase antibodies and adverse reactions to clozapine

SIR,—The use of clozapine has been limited by idiosyncratic drug reactions, especially agranulocytosis.[1] Many such reactions seem to be initiated by reactive metabolites rather than by the parent drug,[2] and since these metabolites usually have short biological half-lives and the target organ in agranulocytosis is the bone marrow, the mechanism might involve formation of reactive metabolites by bone-marrow cells. Myeloperoxidase seems to be the major system in leucocytes that can oxidise drugs to reactive metabolites.[3] When mature neutrophils, monocytes, and some neutrophil precursors are activated, they release myeloperoxidase and generate hydrogen peroxide. This combination, in turn, oxidise chloride ion to hypochlorous acid. Many drugs that are associated with a high incidence of agranulocytosis are metabolised to reactive intermediates by this system.[3] It has been reported that clozapine is metabolised by myeloperoxidase to a reactive free radical,[4] and we have evidence that it is metabolised to a reactive nitrenium ion.[5]

Antibodies against myeloperoxidase have been described in various types of vasculitis[6] and hydralazine-induced lupus.[7] We therefore sought evidence of these antibodies in patients with clozapine-induced adverse drug reactions. We obtained serum samples from 6 patients from the Netherlands with clozapine-induced agranulocytosis while they were still neutropenic. We also investigated a patient at Sunnybrook Health Science Centre.

A 53-year-old woman presented with an 18-year history of chronic paranoid schizophrenia. Her symptoms had been refractory to numerous neuroleptic trials. In the year before admission, facial movements compatible with tardive dyskinesia had arisen. Due to the unremitting psychotic symptoms and signs of tardive dyskinesia, a trial of clozapine was proposed. Perphenazine and flupenthixol decanoate were tapered and discontinued. Clozapine was given, starting at 25 mg daily (day 1, figure), and increased by 25 mg daily.

On day 15 fever (38·5°), diaphoresis, myalgia, arthralgia, and urticarial plaques on elbows, knees, and buttocks developed. Clozapine was stopped, and diphenhydramine was given for