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SHORT REPORTS

Systemic chimerism in human female recipients of male livers

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We have previously reported data from clinical and laboratory animal observations which suggest that organ tolerance after transplantation depends on a state of balanced lymphodendritic cell chimerism between the host and donor graft. We have sought further evidence to support this hypothesis by investigating HLA-mismatched liver allograft recipients.

9 of 9 female recipients of livers from male donors had chimerism in their allografts and extrahepatic tissues, according to in-situ hybridisation and molecular techniques 10 to 19 years post-transplantation. In 8 women with good graft function, evidence of the Y chromosome was found in the blood (6/8), skin (8/8), and lymph nodes (7/8). A ninth patient whose transplant failed after 12 years from recurrent chronic viral hepatitis had chimerism in her lymph nodes, skin, jejunum, and aorta at the time of retransplantation.

Although cell migration is thought to take place after all types of transplantation, the large population of migratory cells in, and the extent of their seeding from, hepatic grafts may explain the privileged tolerogenicity of the liver compared with other organs.

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The hypothesis that acceptance of whole organ allografts depends on the exchange of a special leucocyte population between the donor organ and recipient with subsequent chimerism in both[1] was tested in 9 women who received livers from ABO identical but HLA mismatched male donors. Prednisone doses were 0–15 mg/day and maintenance baseline immunosuppression was with azathioprine in 5 patients, cyclosporin in 3, and nothing in a non-compliant ninth patient whose hepatic graft was failing 12 years after transplantation and 7 years after stopping cyclosporin.

Tissue collection in the 8 patients under continuous immunosuppression consisted of a needle biopsy of the liver, a small piece of skin excised from the groin, and an inguinal lymph node; blood samples were also taken. To avoid genetic contamination, the biopsy of the liver allograft was completed in a separate room with different instruments than used for the skin and lymph-node biopsies. The patient who had stopped cyclosporin was the only one whose liver function was abnormal. However, the allograft removed at her hepatic retransplantation in April, 1992, was found to have been destroyed by recurrent hepatitis C virus rather than by rejection. At this operation, multiple tissue specimens were obtained, including portions of jejunum from the roux-en-Y biliary reconstruction and an ellipse of her aorta removed for a Carrell patch arterial reconstruction.

Fluorescent in-situ hybridisation in the formalin-fixed female recipient tissues was done with cloned, biotinylated DNA probes directed against satellite DNA sequences specific for the Y chromosome (Oncor, Gaithersburg, Maryland, USA). Tissues of known sex served as controls. In addition, the male allograft liver was taken as a fixation and digestion control in each run. Tissues were examined and photographed using ASA 800/1600 colour reversal film on a Nikon Optiphot II epifluorescence microscope.

For polymerase chain reaction (PCR) studies, oligonucleotides specific for the satellite region of the Y chromosome centromere Y-A[2] and for the sex-determining region of the Y chromosome[3] were used as primers to determine the presence of male DNA in the female recipient tissues. After Southern blotting, the amplified material was hybridised to radioactively labelled Y-specific probes to confirm the specificity of the bands visualised in an agarose gel after ethidium bromide staining. As with in-situ hybridisation, the allograft liver samples were invariably strongly positive for the Y probes.

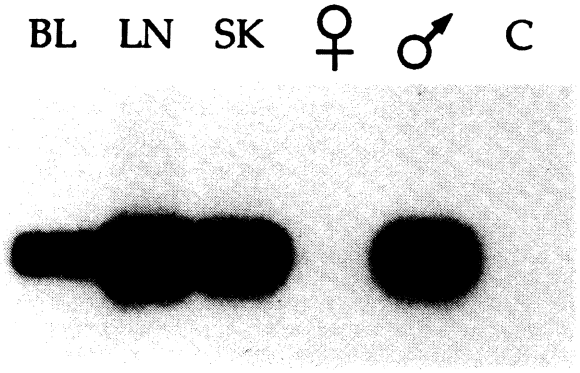
MICROCHIMERISM ACCORDING TO Y CHROMOSOME DETECTION WITH IN-SITU HYBRIDISATION OR PCR

Date of (age at) transplant*	Tissue distribution						
	Liver allograft		Blood	Lymph node		Skin	
	In-situ hybrid-isation	PCR	PCR	In-situ hybrid-isation	PCR	In-situ hybrid-isation	PCR
1. 21/1/76 (30)	+ + +	+ + +	+	+	−	+	+
2. 26/2/78 (5)	+ + +	+ + +	+	+	+	+	NT
3. 9/3/80 (29)	NT	+ + +	−	NT	−	NT	+
4. 4/1/78 (2)	+ + +	+ + +	+	+	+	+	NT
5. 21/3/80 (34)	+ + +	+ + +	+	−	−	+	+
6. 18/2/73 (3)	+ + +	+ + +	−	−	+	+	+
7. 28/2/82 (45)	+ + +	+ + +	+	+	+	+	+
8. 9/9/79 (35)	+ + +	+ + +	+	+	+	+	+
9. 29/8/80† (28)	+ + +	+ + +	NT	+	−	−	+

*All postoperative studies completed in April–June, 1992.
†This patient also tested positive in intestine with in-situ hybridisation and PCR, and in the aorta with PCR.

Hepatocytes, bileduct epithelium, and endothelium of allografts stained positive for hybridisation (table); this result was expected because these cells are known to retain donor specificity.[4] The signal varied from case to case and between different cell types. Under oil immersion, the probe produced a linear-reticular fluorescence characteristic of the Y chromosome at the edge of the nucleus in about 60% of hepatocytes. However, round-shaped signals were not uncommon. In each case, it was important to compare the signal in the liver with the cells detected in recipient tissues. To identify donor cells in the skin often required extensive searching; they were frequently located in stromal tissue near epithelial cells of the epidermis, hair follicles, or adnexal structures, in the peri-adventitial sheath of arteries, or around small superficial dermal blood vessels. Positive cells in lymph nodes were detected in the capsule adjacent to blood vessels and in lymphoid stroma.

All 9 recipients were chimeras according to PCR, and usually in more than one site (figure). Amongst well patients, blood chimerism was found in 6 of 8, skin chimerism in all 6 tested, and lymph-node chimerism in 5 of 8 (table). In the ninth patient, who lost her graft after 12 years because of recurrent viral hepatitis, donor DNA was found in the skin, lymph nodes, jejunum, and aorta sampled at the time of retransplantation (table). At this time, she had not taken cyclosporin for 7 years.



PCR demonstration of chimerism in blood, lymph nodes, and skin of patient 8.

Genomic DNA obtained after a Y-specific amplification of liver, blood (BL), lymph node (LN), and skin (SK) was run on an agarose gel and then Southern blotted to a nylon membrane. DNA from the blood of an unrelated female (♀) and an unrelated male (♂) was also amplified and added as negative and positive controls. The control for PCR amplification (C) was added in the sixth lane of the gel. The liver specimen was not included in the gel because the signal generated from liver DNA, blotted in the same quantity as the other samples, was so intense that it would cover the other specific bands. To test the sensitivity of our procedure, the positive control contained male DNA diluted into female DNA at a ratio of 1 in 10 000.

Peripheral blood lymphocytes from 4 of 9 patients in this study were tested for in-vitro proliferative responses to mitogens (concanavalin A and phytohaemagglutinin) and with third-party mixed lymphocyte culture (MLR). All samples had proliferative responses in the normal range. Although donor cells were not available for MLR testing, many or most of these long survivors undoubtedly have stable donor-specific non-reactivity (tolerance) that is no longer drug dependent. Of 43 liver recipients whom we have followed for $10\frac{1}{2}$ to $22\frac{2}{3}$ years, 7 discontinued immunosuppression 6 to 18 years ago without subsequent rejection.[5] The ultimate step of drug weaning is being contemplated in other patients still receiving immunosuppressive therapy.

The chimerism that has been found invariably in all long-surviving liver recipients[5] seems to involve the movement of self-renewing cells from tissues to blood and vice versa. Donor DNA was found frequently in the blood of late-surviving patients in our study. Donor DNA 26 months after transplantation in the sternum of a recently reported liver recipient whose diagnosis was Gaucher's disease has shown that the bone marrow can be one of the intermediary sites in cell movement.[6]

Although we believe that similar chimerism is the basis for the acceptance of all whole-organ transplants,[1,7] the disproportionately heavy endowment of hepatic grafts with potentially migratory dendritic cells, macrophages, and other leucocytes of bone marrow origin may help explain why the liver is more tolerogenic than other organs.[8,9]

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