osmolality coupled to the large fraction of cardiac output that constitutes the splanchnic-hepatic circulation minimizes shifts in external osmotic pressure, aside from those arising from pathological conditions. It also seems that any osmotic imbalance arising from cell metabolism or transport would be limited to specific cells, given the heterogeneity of metabolic functions in the hepatic lobule. Considering the large degree of intercellular communication between hepatocytes, presumably by means of gap junctions, it is questionable whether such osmotic imbalances could be sustained long enough to effect changes in protein synthesis. Second, extrapolation of the authors' linear plots of protein synthesis vs. time did not intersect the origin; instead they crossed the x-axis at 6 to 7 min. This could reflect diffusion delays. However, it also suggests that activation of a secondary cellular mechanism affects protein synthesis, other than change in cell volume, which occurs rapidly in isolated rat hepatocytes (2). It will be important to consider whether changes in activity or concentration of ionic or organic mediators affect protein synthesis, in addition to changes in water volume *per se*.

In summary, this paper presents new and exciting results showing that changes in hepatocyte hydration affect protein synthesis. This, along with other studies (3), suggests that changes in hepatocyte volume profoundly alter macromolecular synthesis and degradation. Nevertheless, a crucial question remains to be answered: is this phenomenon of importance in normal liver function and pathophysiology, or does it result from experimental conditions triggering highly conserved, vestigial adaptations by cells to survive the dilute milieu of the Precambrian environment (4)? Regardless of the answer to this question, readers and investigators must be aware that, although this problem may be new to hepatologists, these hepatologists are sailing into well-charted waters. There is a rich history to the study of the molecular organization of cells, state of cellular water and effects that changes of organization and hydration have on cellular metabolism (5-7). This field has been rather controversial; notwithstanding, one thing seems quite clear, as aptly stated by Clegg (7): "Although dimly perceived at present, it appears that living cells exhibit an organization far greater than the current teachings of cell biology reveal."

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MIXED CHIMERISM AFTER TRANSPLANTATION: MECHANISM OR MARKER OF SPECIFIC TOLERANCE?

Starzl TE, Trucco M, Zeevi A, Kocova M, Ilstad S, Demetris AJ, Ramos H, et al. Systemic chimerism in human female recipients of male livers. Lancet 1992; 340:876-877.

ABSTRACT

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.

Nine of nine 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.

COMMENTS

Donor-specific immune tolerance has been a persistent and elusive goal in clinical transplantation and thus a focus of intense immunological research since its recognition nearly 50 yr ago. Current immunosuppressive regimens, though clearly effective with the introduction of potent medications such as cyclosporin A and FK-506, suffer from their nonspecific down-regulation of immune surveillance and substantial side-effect profiles. The promise of therapies capable of selectively eliminating host immune responses to the allograft while allowing a normal immune barrier to potential infectious agents therefore remains attractive.

Transplantation of allogeneic livers has been accompanied by several clinical observations with regard to the recipient immune system (1). The incidence of hyperacute rejection caused by preformed cytolytic antibodies is dramatically reduced in liver allografts compared with allogeneic kidney or heart transplants. More important, after liver transplantation skin and solid organs from

the same donor are not rejected, though allografts from an unrelated third party fail in the absence of immunosuppressive medication. Liver transplantation is also associated with reduced incidence of graft-vs.-host reactions despite the large numbers of lymphocytes transferred with the liver allograft. Each of these features indicates that the transplanted liver interacts with the host immune system in some manner so as to create a more tolerant environment. A variety of mechanisms have been advocated to explain these observations, including induction of peripheral tolerance by secretion of soluble major histocompatibility complex class I molecules from the allograft (2), altered secretory patterns by the host antigen–presenting cells (3), clonal deletion of reactive lymphocytes by the allograft (4) and development of suppressor cells derived from the host hematopoietic cell line in response to establishment of stable systemic mixed chimerism after transplantation (5). The article under review seeks to bolster last hypothesis by providing evidence of stable mixed chimerism in the peripheral tissues of long-term liver allograft recipients.

Indirect evidence of systemic mixed chimerism after liver transplantation has mounted over the last decade. The allograft is invaded by host mononuclear cells, which replace the Kupffer cells of donor origin. Thus in the vascular compartment of the allograft mononuclear cells of the host coexist with endothelial cells and hepatocytes of donor origin. Ramsey et al. (6) reported in 1984 results from 40 patients who received ABO-mismatched liver allografts. Twenty-eight percent of evaluated patients demonstrated antibodies to recipient A or B antigens 8 to 16 days after transplantation, persisted for 2 to 4 wk and resulted in clinically evident hemolysis in 5 of these patients. The time course and the predominance of IgG-type antibodies were consistent with a secondary immune response derived from primed donor lymphocytes transferred in the lymphatics and lymph nodes of the allograft. More recently, Selby et al. (7) described a patient with type IV glycogen storage disease who had resolution of extrahepatic amylopectin deposits after liver transplantation. Because the enzymatic defect in this condition is universal, cells of donor origin without the defective enzyme must have migrated to peripheral tissues, where the deposits were degraded over time. The resistance of solid organ allografts to rejection appears to correlate with the volume of passenger lymphoid cells able to enter circulation. In a series of studies reviewed by Russell (8), the size of the transplanted tissue and access to the vascular compartment were directly related to transplant tolerance. Although no mechanistic role can be assigned to the donor immune cells in these studies, the results are consistent with the hypothesis that passenger immune cells modulate organ acceptance by the host.

The article under comment presents evidence of systemic migration by donor cells into the peripheral tissues, where they are maintained over long periods of time. Nine female recipients of ABO-matched, HLA-mismatched liver allografts from male donors were evaluated 10 to 19 yr after transplantation for evidence of systemic mixed chimerism. Eight patients were receiving immunosuppressive medication at the time of the study (five were receiving prednisone and azathioprine; three were receiving cyclosporine and prednisone) and had normal liver function. One patient, evaluated at the time of repeat transplantation necessitated by recurrent viral hepatitis, was reported to have been off cyclosporine for 7 yr without evidence clinical or histological of rejection. No information on prior episodes of acute rejection or specific dosing regimens was provided for the other patients. The patients underwent biopsies of skin, inguinal lymph nodes and liver allograft as well as whole blood sampling. The patient who underwent repeat transplantation also had biopsy specimens taken from jejunum and aorta at the time of surgery. These tissue samples were examined with *in situ* hybridization with a probe specific for satellite regions associated with the Y chromosome. The authors found reactive cells, resembling small lymphocytes, in the subepidermal stroma, perivascular sheaths and the pericapsular region of lymph nodes. In addition, specimens were examined by polymerase chain reaction using primers designed to amplify the satellite regions of the Y chromosome. All patients examined had evidence of chimerism in at least two sites on *in situ* hybridization or PCR. Cells of "male" origin were also detected in the aortic and jejunal specimens. Finally, using peripheral blood lymphocytes from four of these patients, the authors demonstrated normal proliferative responses by host lymphocytes on stimulation by mitogens or irradiated lymphocytes from an unrelated third party. Unfortunately, because no lymphoid tissue was preserved from the donors at the time of transplantation, host immune responses to donor-specific lymphocytes could not be evaluated.

The presence of donor mononuclear cells in the peripheral tissues of transplant recipients has been described previously in patients with severe graft-vs.-host disease after liver transplantation. Roberts et al. (9) used restriction-fragment-length polymorphisms to identify the donor as the source of infiltrating mononuclear cells in kidney, spleen and pancreatic specimens obtained at autopsy. A limitation of the Starzl study is its failure to definitively identify the "male" cells as being of donor origin. Adams et al. (10) demonstrated prolonged circulation of donor lymphocytes in surgical patients, including two liver transplant recipients, who received large-volume blood transfusions. In addition, graft-vs.-host disease is a recognized, albeit rare, complication of transfusions during coronary artery bypass surgery. Circulating lymphocytes could have entered the peripheral tissues at this time. However, the large volume of passenger lymphocytes transferred with the allograft would favor this as the source for these cells.

Despite this limitation, the article raises the role of mixed chimerism in the immune tolerance of the liver allograft. Ildstad et al. (5) have shown in experimental murine models of mixed chimerism that even small numbers of donor lymphocytes can maintain transplant

tolerance. Similarly, the concept of microchimerism has been advocated as a mechanism maintaining tolerance in settings where donor mononuclear cells could not be identified. The application of sensitive techniques to identify these cells in biopsy specimens will now allow this hypothesis to be evaluated.

The importance of systemic mixed chimerism in maintaining long-term allograft tolerance is not addressed by Starzl's study. Eight of the nine patients were still taking immunosuppressive medications at the time of their biopsies. No information is provided on the extent of the chimerism observed in the biopsies; nor is it possible to correlate the degree of chimerism with the clinical course of these patients. Interestingly, the authors report seven long-term liver transplant recipients who are apparently off all medications without evidence of rejection, though details are not provided. This highlights the need for future studies detailing the clinical significance of this observation and identifies patients who may provide important information on long-term tolerance in human subjects. Studying the clinical effects of this mixed chimerism will not be an easy task, particularly because liver-transplant recipients secrete large amounts of soluble class I human leukocyte antigen into circulation and have often received multiple blood transfusions before transplantation. Both occurrences have been demonstrated to induce loss of T-cell responsiveness in animal models and human patients awaiting transplantation (2, 11). Nonetheless, the presence and extent of systemic mixed tolerance may serve as a marker of specific tolerance. If so, it may provide a means to rationally adjust the degree of nonspecific immunosuppression these patients need to endure to maintain graft viability while minimizing the risk of infectious complications. Again, careful examination for the presence and extent of systemic chimerism, combined with direct evaluation of specific host-donor immune responses, will be necessary. Should this chimerism accurately reflect the degree of specific tolerance, additional studies may be directed toward therapies that ensure its development in all patients even as the underlying mechanisms continue to be explored. The detection of systemic mixed chimerism by this study should provide a stimulus for future study into this phenomenon and its clinical importance.

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DRUG TARGETING TO THE LIVER WITH BILE ACIDS: THE "TROJAN HORSE" RESURRECTED?

Kramer W, Wess G, Schubert G, Bickel M, Girbig F, Gutjahr U, Kowalewski S, et al. Liver-specific drug targeting by coupling to bile acids. J Biol Chem 1992; 267:18598-18604.

ABSTRACT

Bile acids are selectively taken up from portal blood into the liver by specific transport systems in the hepatocyte plasma membrane. Therefore, studies were performed to evaluate the potential of bile acids as shuttles to deliver drugs specifically to the liver. The alkylating cytostatic drug chlorambucil and the fluorescent prolyl-4-hydroxylase inhibitor 4-nitrobenzo-2-oxa-1,3-diazol-β-Ala-Phe-5-oxaproline-Gly were covalently linked via an amide bond to 7α,12α,-dihydroxy-3β-(ω-aminoalkoxy)-5-β-cholan-24-oic acid. The chlorambucil-bile acid conjugates S 2521, S 2539, S 2567, and S 2576 inhibited Na^+-dependent [^{3}H]taurocholate uptake in a concentration-dependent manner both into isolated rat hepatocytes and rabbit ileal brush border membrane vesicles, whereas the parent drug chlorambucil showed no significant inhibitory effect. The chlorambucil-bile acid conjugates were able to prevent photoaffinity labeling of bile acid binding proteins in rat hepatocytes by the photolabile [^{3}H]7,7-azo derivative of taurocholic acid indicating their bile acid character. The chlorambucil–bile acid conjugate S 2577 was able to alkylate proteins demonstrating the drug character conserved in the hybrid-molecules.

Liver perfusion experiments revealed a secretion profile of the chlorambucil-bile acid conjugate S 2576 into bile very similar to taurocholate compared to chlorambucil which is predominantly excreted by the kidney. 4-Nitrobenzo-2-oxa-1,3-diazol-β-Ala-Phe-5-oxaproline-Gly-t-butylester (S 4404), a fluorescent peptide inhibitor of prolyl-4-hydroxylase, was not transported in intact form from portal blood into bile in contrast to its bile acid conjugate S 3744; about 25% of the peptide-bile acid conjugate S 3744 was secreted in intact form into bile within 40 min compared with less than 4% of the parent oxaprolylpeptide S 4404.