in these rats during or after GM_3 administration.

In the experiment 2 model, GM_3 was injected intravenously. No complications were seen after daily injections for 7 days after transplantation using a dose of 3 mg/kg GM_3. The mechanisms of action of GM_3 on the prolongation of allograft survival were assessed by immunohistochemistry. These studies suggested that the inhibitory activity of GM_3 resulted from significant suppression of macrophages and cytotoxic T lymphocytes, thereby supporting the results of Hoon et al. and others showing that GM_3 inhibits both IL-2 and IL-2 receptors (*2, 7, 8*). GM_3 might have some unique characteristics as an immunosuppressive agent in organ transplantation. Bremer et al. studied the incorporation of exogenously added GM_3 into Swiss 3T3 cells in vitro (*15*). The data revealed that GM_3 was absorbed by the cell surface and incorporated into the cell membrane after 48 hr. Thus, when GM_3 is injected directly into the transplanted organ, it may be absorbed into the tissue, which reduces the susceptibility to immune attack. It may be possible to reduce the incidence of systemic infection with local administration of GM_3 and also to suppress the immune response during preservation using a solution containing GM_3.

In addition, GM_3 is a molecule present in vivo as a natural substance. Therefore, toxicity would be much less than that of conventional immunosuppressants. Furthermore, Sugimoto and Ogawa demonstrated a method for synthesizing GM_3 chemically (*16*). Thus GM_3 could be used in conjunction with conventional immunosuppressants, thereby reducing the required doses and preventing severe complications after transplantation.

In conclusion, the present study suggests that GM_3 has a significant immunosuppressive effect on transplanted hearts and that it may be a useful immunosuppressive agent for organ transplantation.

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DENDRITIC CELL REPLACEMENT IN LONG-SURVIVING LIVER AND CARDIAC XENOGRAFTS[1]

We described recently how immunosuppression with FK 506 alone markedly prolonged the survival in rats of nonarterialized orthotopic hamster liver xenografts, but not heart xenografts revascularized in the abdominal cavity (*1*). We report here that addition of splenectomy to FK 506 prolonged survival of heart grafts from a mean of 3 days in untreated controls (n=6) to >100 days (7 of 7), permitting us to determine with both organs if the donor dendritic cells were replaced. Male LVG hamsters

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(120–150 g) and male Lewis rats (240–280 g) were used as donors and recipients, respectively. The transplant procedures were performed under methoxyflurane anesthesia in the same way as described before (*1*), except that the heart recipients underwent contemporaneous splenectomy.

Liver recipients were given 1 mg/kg/day intramuscular FK 506 (Fujisawa Pharmaceutical Co., Osaka, Japan) for 30 days and 0.5 mg/kg every other day for another 70 days, terminating treatment on day 100. The splenectomized heart recipients were treated with 2 mg/kg/day FK 506 for 30 days, and thereafter were treated the same as the liver recipients up to 100 days.

Survival of the liver recipients was prolonged with FK 506 from 7.0±0.5 days in untreated controls (n=8) to 66.7±57.9 days (n=10), with 3 of the recipients surviving more than 100 days. The main cause of delayed death under treatment was obstruction of the bile duct, usually with minor histopathologic findings of cellular rejection. After FK 506 was withdrawn at 100 days, the 3 surviving animals remained clinically well for 2 months, at which time liver biopsies showed cellular infiltrates. One animal died after biopsy, but the other 2 lived for an additional 84 and 109 days without therapy before their xenografts were slowly rejected. The 7 heart recipients who survived for 100 days are alive with beating hearts after a further 15–20

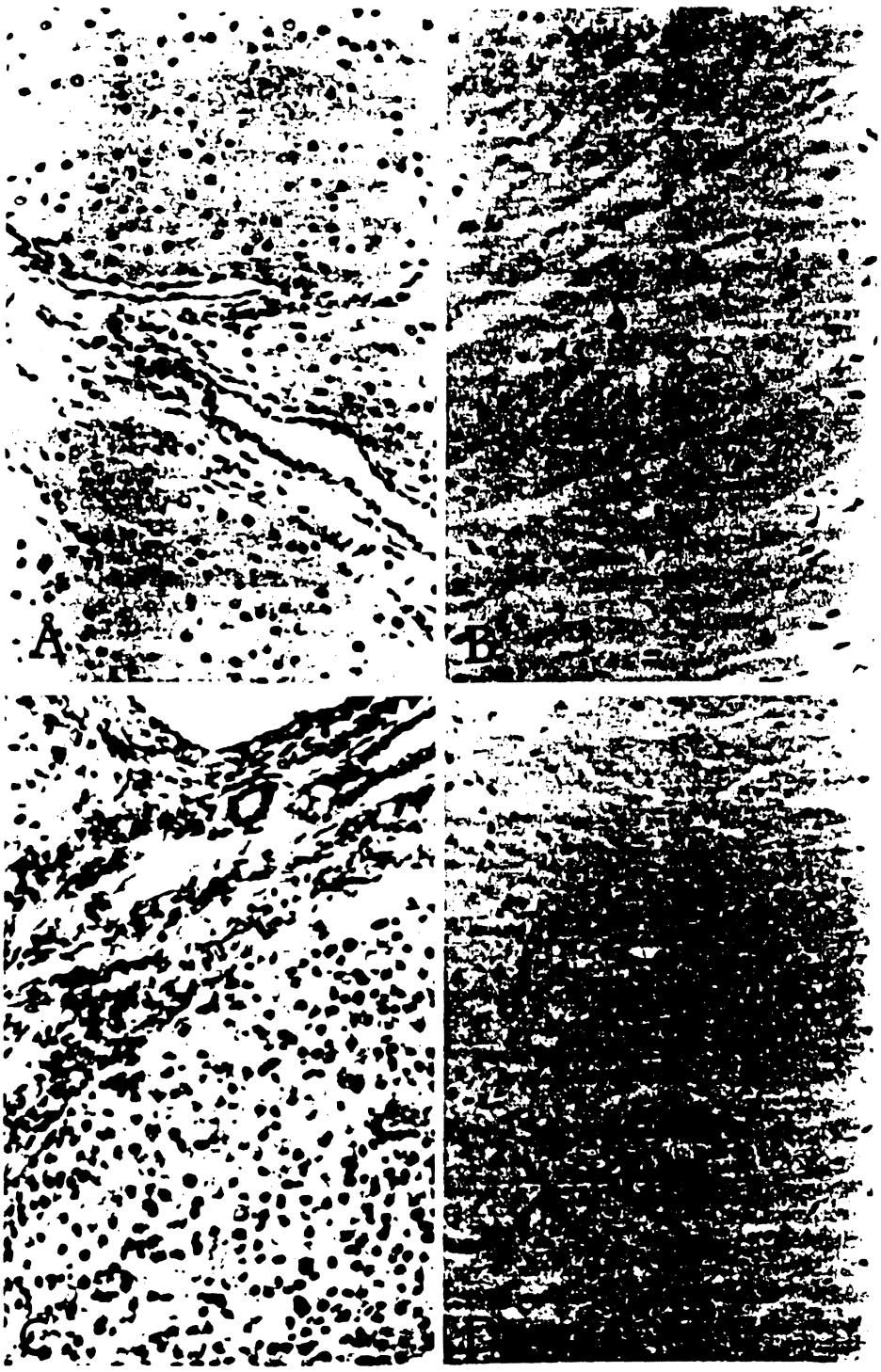

FIGURE 1. Hamster liver (A) and heart (B) in native state and 100 days after transplantation to rats (C and D). Immunoperoxidase stain with L-21-6 mAb and hematoxylin counterstain. Original magnifications: top, ×300; bottom, ×120.

days off therapy.

Liver xenografts were sampled during treatment at 3 (n=3), 30 (n=3), and 60 (n=3) days and beyond the 100-day treatment period (n=3), whereas cardiac xenografts were examined only on post-transplant day 100 (n=3). Tissues were studied with a previously described standard 3-step avidin-biotin complex (ABC) immunoperoxidase technique using the L-21-6 mouse mAb that recognizes class II antigens in Lewis rats (*2, 3*) and a variety for other inbred rat strains. No staining for L-21-6 was detected in normal hamster liver, heart, or spleen (3 samples each).

In contrast, by day 3 after liver transplantation, occasional spindled and round sinusoidal and portal cells were L-21-6 positive in all samples. After transplantation, bile ducts, hepatocytes, and vascular endothelial cells remained L-21-6 negative (donor type) throughout (Fig. 1A). From day 30 onward, whether the grafts were histologically normal or contained a mononuclear portal infiltrate, most of the portal inflammatory cells, sinusoidal Kupffer cells, dendritic-shaped cells in the triads, and spindle cells beneath hepatic venules and in the capsule were L-21-6$^-$ and thus of recipient origin. Although most of the infiltrative cell populations were L-21-6$^+$, 3 distinct cell subgroups could be distinguished based on location, immunophenotype, and morphology. L-21-6$^+$/ED2$^+$ cells with abundant cytoplasm located in the sinusoids and at the edge of the triads were most likely of macrophage lineage. L-21-6$^+$/OX33$^+$ small round cells in the triads, arranged in nodules, were probably B cells. Finally, L-21-6$^+$/ED2$^-$ dendritic shaped cells present in the center of the triads, often near bile ducts, are most likely dendritic cells.

By day 100, the number of L-21-6$^+$ cells in the portal tracts had reached their maximum. Cardiac xenografts sampled on day 100 also had intensely L-21-6$^+$ (recipient origin) spindle-shaped dendritic reticulum cells scattered throughout the interstitium and around arteries (Fig. 1B). These observations were constant in all liver and heart samples with minimum variability.

Cell replacement in the liver allografts was originally observed by K. A. Porter in human liver allografts many years ago (*4, 5*) and was noted recently by Yamaguchi et al. (*6*) in a hamster to rat hepatic xenograft that had survived for 83 days. The findings in the liver and heart xenografts reported here support the contention that dendritic cell repopulation is generic to the acceptance of all grafts (*7*). It is noteworthy that repopulated hepatic and cardiac xenografts had prolonged survival after stopping treatment before they were slowly rejected.

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HUMAN CD8$^+$ XENOREACTIVE T CELLS MEDIATE TISSUE INJURY IN VIVO

There has been longstanding interest in the possibility of transplanting organs or tissues from other species into man (*1*). Studies of human xenogeneic T cell immune responses have demonstrated that the generation of the cytotoxic response involves, as in allogeneic responses, primarily CD4 helper and CD8 cytotoxic T cells (*2–4*). The specificity of xenogeneic antigen recognition by human cytotoxic T cells for polymorphic determinants of antigens encoded by the MHC of another species is comparable to that of alloantigen recognition (*2, 3, 5*), and can be assessed perhaps most easily in human antimouse responses due to the ready availability of murine target cells with defined antigen expression. The human T cell subsets responsible for mediating xenogeneic or allogeneic tissue destruction in vivo remain unclear, with evidence for contributions by both CD4$^+$ and CD8$^+$ T cells and NK cells in the latter (*6, 7*). In these studies, we have investigated human T cell effector function in vivo, using characterized human antimouse effector cell populations adoptively transferred into murine hosts.

Human antimurine effector cells of defined antigen specificity were obtained from a secondary mixed lymphocyte culture with C57BL/6 cells as stimulators and human peripheral blood cells as responders. Human antihuman alloreactive lines were similarly obtained with repeated stimulation of human responder cells from the same donor by an EBV-transformed B cell line carrying both class I and class I disparities as compared to the responder. The alloreactive line 302 and the xenoreactive lines 124 and 159 were obtained by limiting the dilution of a