Methods: We performed 36 procedures of orthotopic liver transplantation in rats, and obtained tissue specimens postoperatively for up to 6 months from the hepatic hilus approximately 10 mm distal to the portal veins anastmosis. The specimens were processed for immunohistochemical stainings (PGP 9.5 and GAP-43) and electron microscopic studies to examine changes in both the immunoreactivity and the ultrastructure of neuronal axons.

Results: Nerve fibers of the specimens stained positive for PGP 9.5 throughout the entire study period, while the immunoreactivity for anti-GAP-43 antibody was negative in those obtained at 1 and 2 POD. GAP-43-positive neurons were first observed to appear at 3 POD. The immunoreactivity increased to the maximum in 5–7 days, remained the same until 2 weeks, and then gradually returned to the control level at and after 4 months post-transplantation. Electron microscopic examination at 3 POD revealed that there were many regenerating axons present among degenerating axons in the nerve bundles.

Discussion: GAP-43 is known as an excellent marker of axonal growth of neurons in the regeneration process. Results of the present study suggested that the extrinsic hepatic reinnervation began shortly after liver transplantation with regenerating axons reaching to the hepatic hilus in as early as 2–3 days, and almost terminated in 3 months. It was considered that those regenerating axons grew into the spaces of existing nerve bundles of the graft liver choosing these spaces as the regeneration pathway.

PP76

Allotransplantation of Hepatocytes into the Spleen Following Liver Plus Kuppfer Cells Inoculation into the Thymus

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Introduction: Some kinds of allo-antigens when inoculated into the thymus have induced specific immunotolerance for subsequent allo-transplants (kidney, heart). However, this technique had failed when using allo-hepatocytes. Following the experience of other groups, we tried to improve our results by treating the animals with a very short course of immunosuppressants two days prior to thymus inoculation; and thus, allohepatocytes surviving in the spleen could be found in a few animals. Some authors suggested that isolated hepatocytes fail to express their antigens, and thus immunotolerance is not achieved. We have incorporated Kuppfer cells (which act as antigen presenting cells in the liver) to the thymus inoculum.

Methods: Syngeneic male Wag rats weighing 250 g. (donors) or 75 g. (recipients) have been used. Hepatocytes and Kuppfer cells have been obtained by standard collagenase digestion of the liver. Surgery was performed under ether anesthesia. Through a midline incision, 3×10^6 hepatocytes plus 3×10^5 Kuppfer cells were inoculated into each lobe of the thymus (0.2 ml). Fifteen days later, hepatocytes were inoculated into the spleen: a) 8×10^6 cells in 10 rats, sacrificed on day 7th; b) 20×10^6 cells in 14 rats, sacrificed on day 7th; (7 rats) and 14th (7 rats). The survival of hepatocytes both in thymus and spleen was assessed by two independent observers on hematoxiline-eosine stained sections.

Results: A relevant number of hepatocytes were found in the thymus of every animal. However, in the spleen, though some hepatocytes could be identified in all of the animals, their number was low; only in three animals we could found hepatocytes in numbers similar to those seen after syngeneic inoculation. In any case, cordonal organization of hepatocytes could never be seen.

Discussion: The presence of hepatocytes in all of the spleens may be due to a wicker rejection. Perhaps 15 days is not time enough to repopulate the animal with lymphocytes grown up in the thymus in the presence of the new antigens.

Conclusion: Specific immunotolerance for hepatocyte transplantation could be obtained by intrathymic antigen inoculation; though effectiveness of the technique has to be enhanced.

PP77

Variable *Ex Vivo* Whole Blood Isolated Lung Perfusion System

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Introduction: *Ex vivo* hemoperfusion of whole organs is a new experimental technique to study mechanisms of reperfusion injury or rejection. In transplantation medicine, genetically modified animals are being developed for clinical use. Our group developed a model for *ex-vivo* hemoperfusion of kidneys up to 6 hours and reported first early changes of genetically modified kidneys perfused with whole