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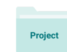

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ontogenesis. These hESC derived cells will further the study of liver development and have the potential to become a reliable source of cells for hepatocyte transplantation and bioartificial livers.

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513 TLR3 SIGNALING ATTENUATES LIVER REGENERATION

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Background and Aim: The liver has a remarkable capacity to regenerate after injury or partial hepatectomy. The current model states that cell damage triggers TLR signaling leading to the induction of NF-kB and secretion of inflammatory cytokines, such as IL-6. IL-6 was previously shown to prime the liver regeneration process. TLR3 is unique among TLRs in that it signals via TRIF and not via MyD88. TLR3 signaling may lead to activation of the inflammatory or the apoptotic pathways. The inflammatory pathway is mediated mainly by Rip1 and leads to NF-kB activation, whereas the apoptotic pathway, believed to be mediated by Rip3, leads to the inhibition of NF-kB activation by Rip1, resulting in caspase-8 activation.
Methods and Results: We explored the role of TLR3 in liver regeneration which was induced by performing 70% partial hepatectomy (PHx) on 3-months old C57Bl (background) or $TLR3^{-/-}$ mice. We show that in the absence of TLR3, hepatocyte growth was accelerated. Furthermore, in $TLR3^{-/-}$ mice, IL-6 and soluble IL-6 receptor levels were lower than in background mice, suggesting that the differences in liver regeneration rates are independent of IL-6. Analysis of TLR3 signaling following 70% PHx revealed significantly higher levels of activated NF-kB in $TLR3^{-/-}$ mice at the early 4 hrs time point and conversely, lower levels after 24 hrs. In accordance, at 4 hrs, the levels of Rip3 and of activated caspase-8 were lower in $TLR3^{-/-}$ mice compared to background mice.
Conclusion: We suggest that within the first hours following PHx, TLR3 is activated, leading to an increase in Rip3 which on the one hand inhibits NF-kB activation, and on the other hand activates caspase-8, consequently resulting in a delay in cell proliferation. Thus we propose that following PHx TLR3 signaling inhibits the initiation of liver regeneration by blocking NF-kB activation and inducing the apoptotic pathways.

514 ULTRASTRUCTURAL ASPECTS OF LIVER REGENERATION IN CHRONIC HEPATITIS C PATIENTS

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Background and Aim: The liver has a unique ability to regenerate after loss of mass and function such as following surgical resection or toxic liver injury. Electron microscopy is an accessible and credible tool for evaluation of the hepatic structure and adaptation. This study aimed to investigate the most common intracellular changes reflecting the liver regenerative capability in case of chronic hepatitis C. The ultrastructural peculiarities of hepatocyte regeneration were assessed by transmission and scanning electron microscopy. Additionally, changes of sinusoidal endothelial cells and hepatic stellate cells located in the close vicinity to the hepatocytes with ultrastructural signs of intracellular regeneration were studied.
Material and Methods: Electron microscopy was carried out on bioptates obtained from 34 patients with chronic hepatitis C; ages ranged 26–45 years.
Results: The overall analysis of the hepatocyte external contour detected severe loss of the microvilli at the vascular and bile pole of the cell detected by use of both transmission and scanning electron microscopy. Smoothening of contour at the hepatocyte bile pole was accompanied by rearrangement of actin and intermediate filament cytoskeleton. Thickening and ring-like appearance of actin filaments was a common finding. Distribution of mononuclear cells comparing to binucleated was not very much different from the other pathological settings. Mitochondria greatly varied in number and showed a polymorphous appearance but summarization of the mitochondria changes suggested that these were present in an increased number in so called "dark cells" whereas a decreased amount reflected "light cells". In a majority of cases small (except the alcoholic damage) and dense mitochondria, sometimes with inclusions, and without an alteration of the external membrane were noticed. Aggregates of cristae were not common. Proliferation of smooth endoplasmic reticulum next to the lipid droplets and lipid cysts was very common finding that usually had a local character rather than throughout the lobule.
Conclusions: Ultrastructural assessments, in general, and mitochondrial analysis, in particular, have clinical relevance. Reactive mitochondrial changes are the first evidences of cell stress and death. Examination of plastic sections and fractionation technique can provide useful information about the liver tissue.

515 INSULIN PRODUCTION AND PANCREATIC GENE EXPRESSION ANALYSIS IN HUMAN FETAL LIVER PROGENITOR CELLS

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Introduction: Beta cell destruction or insufficient insulin production are the hallmarks of diabetes mellitus (Type 1 diabetes). Pancreas transplantation or islet cell implantation efficiently restores normolgycemia, but requires life long immunosuppressive therapy and are limited by tissue supply. Self renewal and transdifferetion capacity of adult stem cells promises to solve the problem of cell shortage. Recently, several studies have demonstrated that hepatic stem cells can transdifferentiate to pancreatic cells and vise-versa. The present study was carried out to check the presence of pancreatic precursor in human fetal liver. The expression of Pancreatic genes such as insulin, glucagon, somatostatin, and Glut-2 were analyzed in isolated human fetal liver cells and the production of insulin was assessed in invitro culture.
Method: Fetuses (14–20 weeks) were collected as a result of spontaneous abortions with the from the Maternity Hospital. Isolation of liver cell suspension was done by collagenase digestion method. The viability of the cells was checked by TBE. Cells were sorted using CD326 antibodies by Magnetic cell sorter. The sorted cells were cultured with different concentrations of glucose (5mM to 30mM) in DMEM/HAM'SF-12. The amount of insulin production was estimated in the cell culture. After every 24 hours the production of insulin was measured in the media for 7 days by the method of RIA.
The amount of C-peptide production after 12 hours of glucose induction was measured for every 2 hours interval in the media by the method of RIA.Total RNA was isolated from sorted cells using Trizol cDNA was constructed to study the pancreas specific gene expression. PCR programme consists of 35 cycles PCR products were separated by electrophoresis in 1.5% agarose gel.
Results and Conclusion: The data obtained from this study revealed that the hepatic progenitor cells expressed almost all the genes of pancreas. The production of insulin in sorted cell cultures increased upto 7 days which indicates that the cells were transdifferentiating into insulin producing cells. C-peptide secretion was high in glucose induced cells. Hence liver

cells could be used as one of the source of pancreatic islet cells for the treatment of type I diabetes.

516 EXTRA-CELLULAR MATRIX REMODELLING IS CRITICAL TO THE OVAL CELL RESPONSE AFTER CHRONIC LIVER INJURY

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Background and Aims: In advanced cirrhosis hepatic regeneration can occur via an intrinsic facultative stem cell compartment located within the terminal branches of the biliary tree. Hepatic progenitor cells (oval cells in mice) are found in close anatomical approximation to myofibroblasts and macrophages, within their stem cell niche. Stem cell fate may be determined by constituents of the extra-cellular matrix, such as collagen I and laminin, as well as by growth factors secreted by the supporting mesenchymal cells. The aim of the study was to determine how the distribution of collagen I and laminin influences the oval cell response after iterative carbon tetrachloride injury (CCl4) in a mouse model of chronic liver disease.
Methods: The r/r collagen transgenic mouse (r/r) expresses mutated collagen I resistant to collagenase degradation, has an exaggerated fibrotic phenotype to CCl4 liver injury, and shows impairment of collagenolysis post injury. Liver fibrosis was induced by 8 weeks of repeated intraperitoneal CCl4 in r/r mice and wild-type (Wt) C57B6 controls (n = 12 each). Liver tissue was harvested at days 1 & 9 post injury. Immunohistochemical analysis of histological sections was performed for laminin, oval cells (pancytokeratin), proliferating mature hepatocytes (Ki67), and collagen (sirius red).
Results: r/r mice developed significantly more liver fibrosis than Wt controls, and showed persistence of collagen into recovery. Concomitantly, r/r mice had a markedly attenuated oval cell response throughout the recovery phase (347±57 vs. 80±10 day 1, p = 0.01, and 251±23 vs. 94±24 day 9, p = 0.004, mean no. cells per x400 field +/-SEM), with blunting of oval cell projections into the hepatic lobule on histology. There was no significant difference in parenchymal regeneration via proliferation of mature hepatocytes across all groups (p = 0.10). By day 9 post injury dense laminin deposition around portal tracts and throughout the hepatic lobule can be seen in Wt but not in r/r mice.
Conclusions: Persistence of collagen I and a paucity of laminin deposition after hepatic injury are associated with an inability to mount an oval cell response. Matrix remodelling appears critical to parenchymal regeneration via hepatic stem cells, and laminin-progenitor cell interactions may be an important feature of this.

517 ROLE OF KUPFFER CELL ON HEPATOCYTE PROLIFERATION

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Background and Aims: Kupffer cells produce inflammatory cytokines and promote liver regeneration after hepatectomy. The aim of this study is to investigate the mechanism of hepatocyte proliferation by Kupffer cells in vitro.
Methods: Kupffer cells of male BALB/c mice were obtained by the collagenase perfusion method. Immortalized hepatocytes (TLR2) were used instead of primary cultured hepatocytes. To elucidate the mechanism of the proliferative effect of Kupffer cells, DNA synthesis of hepatocytes was measured under various conditions and the related cellular signals were analyzed.
Results: DNA synthesis was significantly increased in the hepatocytes cultured with Kupffer cells. However, when Kupffer cells and hepatocytes were separated, Kupffer cells did not have a proliferative effect. The supernatant of co-cultured medium of hepatocytes and Kupffer cells also have a proliferative effect. The supernatant of co-cultured medium contained interleukin-6. Ten to 24 hours after incubation of hepatocytes with Kupffer cells, STAT 3 of hepatocytes was activated.
Conclusions: Kupffer cells have hepato-proliferative effect. Direct contact between Kupffer cells and hepatocytes was necessary for the proliferative effect. The STAT 3 pathway was important for proliferation of hepatocytes by Kupffer cells.

518 LIVER ENDOTHELIAL CELLS ENHANCE THE BIOLOGICAL EFFECT OF CXCL9 AND CXCL12 BY UPTAKE AND SURFACE PRESENTATION

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Transmigration through endothelium is a key regulator for the homeostatic balance of T cells and local inflammation mediated by adhesion molecules and chemokines. We demonstrate that the chemotactic effect of CXCL12 and CXCL9 on naive and memory CD4+ T cells is significantly enhanced by liver sinusoidal endothelial cells whereas endothelioma cells and ex vivo-isolated lung endothelia inhibit chemokine-driven transmigration. As to the involved mechanisms chemokine-induced activation of LSEC was excluded by blockage of Gi-protein-coupled signaling and use of knock out mice, respectively. After pre-incubation of CXCL12 to the bottom, but not to the apical side, LSEC enhanced transmigration as efficiently as in presence of the soluble chemokine. Blockage of transcytosis in LSEC significantly inhibited this effect suggesting that chemokines taken up from the basolateral side and presented in close vicinity to adhesion molecules on the luminal side of endothelial cells trigger T cell transmigration. Thus, our findings highlight the importance of liver sinusoidal endothelial cells for the in vivo effects of chemokines under normal conditions and in the inflamed liver.

519 ISOLATION OF HEPATIC STEM CELLS USING A NOVEL SURFACE ANTIGEN;GP38

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Background and Aims: Previously, we clarified the surface antigen profiles of hepatic progenitor cells (HPCs) contained in the E13.5 fetal livers, and isolated them as CD49f+CD45-Thy1-cells using flowcytometry. The HPCs showed the heterogeneous staining pattern of albumin and cytokeratin 19 (CK19), suggesting that different populations of HPCs at various stages of differentiation existed in the fraction of CD49f+CD45-Thy1-cells. In addition, we identified several novel candidates of surface antigens for hepatic progenitor cells by DNA subtraction analysis between mature hepatocyte fraction and hepatic progenitor cell fraction. The aim of this study was to detect and isolate more immature HPCs, or hepatic stem cells, using a novel surface antigen (gp38).
Methods: Livers were harvested from E11.5, E13.5, and E18.5 fetal mice respectively, and digested with collagenage. HPCs were obtained by forming cell aggregates, and divided into two subpopulations using flowcytometry: gp38-positive HPCs (CD49f+CD45-Thy1-gp38+), and gp38-negative HPCs (CD49f+CD45−Thy1−gp38−). We characterized both types of HPCs

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