153 **MECHANISMS DETERMINING AUGMENTATION OF THE TRANSPLANTED HEPATOCYTE MASS WITH GROWTH STIMULATING SIGNALS.**

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Transplanted hepatocytes are permanently incorporated in liver parenchyma and retain normal function. Therapeutic strategies will benefit from a large transplanted hepatocyte mass, which can be achieved when host hepatocytes are ablated. To define whether mitogens could increase transplanted hepatocyte numbers in the normal liver, we first analyzed the effect of partial hepatectomy (PH), which induces coordinated release of growth factors and soluble signals. In one experiment, $2x10^6$ transgenic G26 HBV hepatocytes that produce serum HBsAg, as an excellent reporter of transplanted hepatocyte mass, were injected intrasplenically into congeneic C57BL/6J mice. After cell engraftment for 4 weeks, 50% PH was performed. However, instead of rising, serum HBsAg levels persistently declined in recipients, suggesting no proliferation in transplanted hepatocytes. Similarly, when hepatocytes were transplanted into syngeneic dipeptidyl peptidase IV-deficient (DPPIV-) F344 rats followed 4 weeks later by 68% PH, neither transplanted cell number nor cluster sizes increased. The absence of hyperplasia in transplanted cells was in agreement with our recent findings showing increased hepatocyte maturation, cell ploidy, and senescence-type changes after PH. In contrast, when hepatocytes were transplanted into the liver of DPPIV- recipients immediately after 68% PH, the cluster sizes and number of transplanted cells significantly increased at 14 days (p<0.001). Host hepatocytes showed peak DNA synthesis at 24 hrs after PH, whereas transplanted cells underwent DNA synthesis later (3-5 days), with their number increasing in the second week. This different hyperplastic response indicated that transplanted cells escaped from hypertrophic signals affecting host hepatocytes when injected after PH. To determine whether growth factor stimulation could induce hyperplasia in transplanted hepatocytes, we intraportally infused 2.4 mg/kg/day hepatocyte growth factor (hHGF; Genentech Inc.) by osmotic pumps for up to 1 week in mice, 4 weeks after transplantation of G26 HBV hepatocytes. Alternatively, hHGF infusion was commenced 1 day prior to hepatocyte transplantation in DPPIV- rats. The DNA synthesis rates were increased after exposure to hHGF in cultured primary hepatocytes, as well as intact mouse or rat liver. However, the number of transplanted hepatocytes did not increase in the liver after hHGF infusion. **CONCLUSIONS:** Transplanted hepatocytes can proliferate in the normal liver in response to appropriate stimuli. Nonetheless, growth factor-induced hyperplasia in transplanted hepatocytes will depend largely upon the rate of host hepatocyte losses and ambient mitogenic conditions in the recipient liver itself.

154 FETAL HEPATOCYTES ARE SUITABLE FOR TRANSPLANTATION AND GENE THERAPY.

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Fetal hepatocytes (FH) are thought to proliferate, are less immunogenic and are relatively resistant to ischemic injury. These qualities could enhance FH engraftment, proliferation and gene transfer requiring active DNA synthesis. Only few attempts have been made to transplant FH in rats. Data on retroviral gene transfer in FH from late gestation period are not available. Study #1: FH were isolated from Sprague-Dawley rats at day 20 of gestation using collagenase/DNAse digestion method. Cell isolates contained 87±2% hepatocytes (TEM; FACS using α-fetoprotein and albumin markers). In 10 day cultures, FH expressed both α-fetoprotein and albumin (Northern). In non-stimulated FH, DNA synthesis was greater than that in EGF-treated (20ng/ml) adult cells [(3H)-thymidine uptake: 477671±71650 *vs.* 69883±17500 cpm/mg protein; p<0.01]. In addition, FH were susceptible to transduction by a retroviral vector carrying the *E. coli* lac-Z gene [n=6; 5%-10% of cells stained (+) for X-Gal]. Study # 2: Male Nagase analbuminemic rat recipients were maintained on Cyclosporine A (10mg/kg, i.m., x3/week). S-D rats served as cell donors. In Grp. I (n=7), $2x10^7$ FH were infused into the anterior liver lobes. In Grp. II (n=6), $2x10^7$ FH were infused into the posterior lobes in which regenerative response was induced after 24 hrs by ligation of the portal branch (PBL) supplying the anterior lobes. In Grp. III (n=7), $2x10^7$ adult hepatocytes were infused into the anterior liver lobes. Grp. IV (n=5) and V (n=5) controls received intraportal infusion of saline with/without PBL. Results: Only recipients of adult hepatocytes and FH under a regenerative response (PBL), showed elevation in serum albumin levels (ELISA). In these rats, immunohistochemistry confirmed presence of albumin-positive hepatocytes. Data are shown in mg/dl as means±SD; all p<0.05 (ANOVA).

Group	Baseline	Day 7	Day 14	Day 21	Day 28
I	0.7±0.1	3.2±2.6	2.4±2.4	2.9±2.0	2.9±2.5
II	0.8±0.2	27.6±11.6	18.8±8.2	15.8±6.8	15.3±5.7
III	0.8±0.1	11.1±2.3	26.6±9.3	35.4±6.6	34.1±15.0
IV	0.8±0.1	0.8±0.1	0.8±0.1	0.7±0.1	0.6±0.2

In conclusion: Fetal rat liver from late gestation period is highly enriched with hepatocytes. They are highly regenerative and susceptible to retroviral transduction. Fetal rat hepatocytes can engraft and function in the adult rat liver if transplanted under a hepatic regenerative stimulus.

155 TREATMENT OF FULMINANT HEPATIC FAILURE RATS WITH A BIOARTIFICIAL LIVER (BAL) PROLONGS SURVIVAL AND IMPROVES HGF - c-met - TGFβ1 BALANCE.

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We have previously reported that rats in which fulminant hepatic failure (FHF) was induced by partial (68%) hepatectomy and right lobes necrosis died within 48 hrs without any signs of liver regeneration (Hepatology 1996;24:1452-9). We also found that hepatocyte transplantation prolonged survival and triggered proliferative response in the FHF liver (Hepatology 1996, 24:307A). Here, we show that BAL treatment had similar effects. Inbred male Lewis rats (350g) were used. FHF was induced as described above. The BAL consisted of a hollow-fiber module (Microgon) loaded with $3x10^8$ syngeneic microcarrier-attached hepatocytes. The sham-BAL contained beads only. Grp.I FHF rats (n=12) were treated with the BAL. In Grp.II FHF controls (n=12), sham-BAL was used. At 4 hrs after induction of FHF, the BAL circuit was attached to the femoral vessels. A pump-assisted blood perfusion was then carried out for 4 hrs at 2ml/min. Survival time was determined in 6 rats from each group; the remaining rats were euthanized at 4 hrs after treatment. At sacrifice, blood was collected for measurement of HGF and TGF-β1 levels (ELISA). The native liver was analyzed for HGF and c-met mRNA expression (RNase protection assay). Compared to sham-BAL controls, BAL-treated rats lived longer and had better metabolic parameters (Table; means ± SD; *-P<0.05). In BAL-treated rats, plasma levels of HGF remained unchanged and TGF-β1decreased. The livers of sham-BAL rats showed no HGF and weak c-met mRNA expression, while those of BAL-treated rats were strongly c-met and HGF positive. Body core temperature at 12 hrs was significantly higher in BAL-treated rats than in sham-BAL controls.

Grp.	Survival (hr)	TGF-β1 (ng/ml)	PTT (sec)	PT (sec)	BUN (mg/dl)	Uric Acid (mg/dl)	Alk.Phosph. (U/L)	Rectal t^{0}. (^{0}C)
I	27±5*	16±6*	36±6*	21±2*	27±4*	1.0±0.5	240±123*	32±1*
II	17±2	26±7	55±9	25±1	36±4	2.0±0.8	516±212	28±2

In conclusion: "Pulse treatment" of FHF rats with a hepatocyte-based BAL, prolonged survival time, improved blood coagulation, enhanced expression of HGF and c-met receptor in the native liver and lowered blood levels of a potent hepatic growth inhibitor (TGFβ1).

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156 **LONG-TERM CULTURE OF PRIMARY RAT HEPATOCYTES IN A PACKED-BED BIOREACTOR USING FIBRA-CEL DISCS**

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Primary hepatocytes following isolation from the liver lose many of their differentiated functions and survive *in vitro* for relatively short times. The aim of this study was to determine the feasibility of adapting a large scale cell bioreactor system previously used to culture CHO cells or hybridoma cells in bulk, for use with long term stable functional primary hepatocyte cultures.

Rat hepatocytes (2×10^8) were attached to 3-D polyester fibre discs housed in a 100 ml fixed-bed basket within a 500ml working volume. A recirculating perfusion system with 4 gas pH and disolved O_2 control was used and cultures were maintained for up to 90 days. Viability was assessed by MTT assay, urea and albumin production. Other metabolic and biochemical parameters were monitored and morphology was assessed by EM.

Upon innoculation, cell attachment to discs was almost 100% after approx 2h. MTT cell counts indicated that viability at day 18 had fallen to 68.6% but that this was stable throughout the remainder of the experiment (68% at day 90). Initial albumin production rate was high (5 pg/cell/h) and declined to a steady state (2.2 pg/cell/h) after a few days. Urea output in response to addition of 1mM NH_4Cl was similarly maintained at approx 2 pg/cell/h. Notably these steady states were maintained for > 90 days. Greater stability and output was achieved in the presence of 10% FCS. EM morphology showed good preservation of nuclear and organelle ultra-structure with some flattening of cellular architecture at 90 days. General phase I and phase II reactivity using 7-ethoxycoumarin metabolism was found to be well maintained.

These data indicate that differentiated primary hepatocytes can be maintained in a large scale cell bioreactor system with relative ease and for several months. The experimental design has numerous potential applications in the development of bioartificial liver systems as well as for long term drug metabolism and toxicity studies.