British Association for the Study of the Liver Meeting

The following abstracts were presented at the British Association for the Study of the Liver Meeting, Hammersmith Hospital, London, UK, September 9–10, 1999.

01 Hepatic stellate cells (HSC) express low affinity nerve growth factor receptor (p75) and undergo apoptosis in response to nerve growth factor (NGF) J IREDALE, N TRIM, R ISA, S MORGANS, M EVANS
University Departments of Medicine and Pathology, Southampton General Hospital, Southampton, UK
Following the demonstration that apoptosis of activated HSC contributes critically to recovery from hepatic fibrosis, the mechanisms regulating HSC apoptosis have become an active area of investigation. HSC are known to express Fas and to respond to stimulation with Fas-ligand by undergoing apoptosis. Fas is a transmembrane receptor of the TNF receptor (TNF-R) superfamily, is widely expressed (notably by hepatocytes) and triggers apoptosis via a cytoplasmic death domain. In this study we have examined the hypothesis that activated HSC express p75, a further member of the TNF-R family which has previously been described in neural crest derived cells and dendritic stromal cells in a variety of tissues. P75 has been demonstrated to trigger apoptosis in response to stimulation by ligand. Activated HSC at 7 and 14 days of primary culture were demonstrated by Western analysis and immunostaining, to express p75. In addition, passaged human HSC were also p75 positive by Western analysis. P75 was demonstrated in vivo and immunolocalised to cells with a myofibroblast-like morphology in the in the fibrotic bands of six archived fibrotic and cirrhotic liver biopsies. Immunostaining of parallel sections indicated that these cells were u-SMA positive, identifying them as activated HSC. Activated HSC in fibrotic rat liver (induced by 4 weeks of CCl₄, intoxication) also expressed p75. HSC apoptosis in tissue culture in the presence of 16% serum was quantified with and without 0.1-100ng/ml NGF (the paradigm ligand for p75) by in situ quantifying of apoptotic bodies in tissue culture wells after addition of acridine orange. HSC demonstrated a dose dependent increase in apoptosis in response to NGF, most significant at 100ng/ml (2.5 fold increase 0.05>p>0.01 by Wilcoxon Rank, n = 7) after 24 hours. NGF 100ng/ml had no effect on HSC proliferation, but was associated with a 35% decrease in total HSC DNA relative to controlafter 24 hours (n = 3).
These data demonstrate that activated HSC express p75 and respond to NGF stimulation by undergoing apoptosis. We therefore report p75 is a novel marker for activated HSC and suggest that stimulation of p75 may provide a mechanism for selective apoptosis of HSC.

02 N-acetylcysteine prevents renal failure in a rat model of the hepatorenal syndrome R ANAND, D HARRY, S HOLT, K MOORE Centre for Hepatology, Department of Medicine, Royal Free & University College Medical School (University College London), Royal Free Campus, London, UK
We have recently shown that administration of N-acetylcysteine to patients with early hepatorenal syndrome (HRS) reverse the decline of renal function. However, the mechanism of action is unknown. We have recently changed our protocol of the hepatorenal syndrome based on acute liver failure induced by injection of galactosamine. This model has a marked reduction of creatinine clearance and sodium excretion. Moreover, the kidneys are normal on histological examination and there is a marked reduction of renal blood flow. In this study we have used this model to evaluate the effects of N-acetylcysteine on renal function. Rats (Sprague Dawley) were injected intra-peritoneally with either galactosamine (1.1g/kg) or saline (as sham control). Galactosamine treated with galactosamine had an acute fall in creatinine clearance from 1.04 ± 0.07 ml/min to 0.36 ± 0.03 ml/minute by 48h, p<0.05. The daily administration of N-acetylcysteine (150mg/kg) to the rats, prior to and after induction of liver injury by galactosamine, had no effect on the degree of liver injury (AST 7628 ± 93 U/L). Treatment with N-acetylcysteine completely prevented the development of renal failure (creatinine clearance 1.17 ± 0.1 ml/min v 0.56 ± 0.03 ml/min) p<0.05, and rise in serum creatinine (43 ± 1.5µM vs 65 ± 2µM, p<0.05). Galactosamine induced hepato renal syndrome was also associated with a modest decrease of urinary sodium excretion (1.27 ± 0.05 to 0.86 ± 0.1 mmol/day), and this was prevented by the administration of N-acetylcysteine (1.3 ± 0.18 mmol/day). We conclude that N-acetylcysteine prevents the development of renal failure in a model of acute liver failure and the hepatorenal syndrome. Further studies to elucidate the mechanism of action of N-acetylcysteine are underway.

03 Hepatocellular siderosis and cirrhosis in bovine haemochromatosis: a spontaneously occurring, symptomatic model of human haemochromatosis A P WALKER, D F WALLACE, J S DOOLEY, W C RUSSELL, A P WALKER, K SAEB-PARSY, J S DOOLEY, R WILLIAMS, N V NAOUMOV Institute of Hepatology, University College London, London, UK
Background: Hepatitis B virus (HBV) variants with deletions in the core gene have been described in 7–25% of patients with chronic HBV infection. We have previously reported several common features of HBV variants. They always occur together with wildtype HBV, the deletions are frequently in frame, the deleted fragment does not affect the polymerase gene and the variants do not confer resistance to interferon treatment. So far, the functional characteristics and the biological significance of these HBV variants have not been defined. Aim: To investigate the replication competence and HBV Ag expression of naturally occurring HBV variants with core gene deletion, we chose a strategy where the entire HBV genomes containing the full length core or deletion variants are amplified and without further modifications are expressed in HUH-7 cells. Methods: DNA was extracted from serum of 4 HBsAg positive patients who were previously shown to have HBV core gene deletion variants. PCR for HBV DNA was performed with a particular set of primers which allow the amplification of the full length HBV genome. The amplimers were cloned into pUC19 and a range of clones with full length and deleted core gene were selected and sequenced with an automated sequencer. The HBV DNA was then cleaved from the vector and transfected into HUH-7 cells and the presence of HBV DNA replication intermediates was sought by Southern blot analysis. In order to study the
expression and assembly of nucleocapsid protein, we subsequently cloned the core gene of all clones into a prokaryotic expression vector and core protein synthesis was detected by Western blot. Results: Fifteen HBV DNA clones were sequenced—4 with wild type core gene and 11 deletion variants. The size of the deletions ranged from 63 to 243 nucleotides, all of them located in the central region of the core gene. 9 deletion variants were in frame, 2 out of frame. The nucleotide sequence variation in the entire core gene between clones derived from the same patient varied from 0 to 1.1% (on average 0.3%), thus indicating the close relationship between the clones and the deleted variants in each patient. Southern blot analysis indicated that some of the clones with a core deletion can produce HBV DNA replicative intermediates. Western blot analysis of the core protein expressed in E. coli showed efficient translation from some of the variants with an in-frame deletion resulting in shorter core proteins. Conclusion: The amplification of the full length HBV genomes allows characterisation of core deletion variants as they exist in patients with chronic HBV infection. Our data suggest that some of the naturally occurring HBV variants with a deletion in the core gene express core protein and may be replication competent without the help from the wildtype HBV.

05 Adoptive transfer of HBcAg-specific CD4+ T cells is associated with HBsAg clearance in Chinese chronic HBV carriers

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Institute of Hepatology, Department of Biology and Anthropology, University College London, London, UK

TTV is a newly identified human DNA virus with a high prevalence in the general population worldwide. TTV has unusually high variability and three major genotypes, with greater than 30% nucleotide divergence, have been identified. The cells in which TTV replicates and the pathogenicity of this virus remain unclear. The aim of this study was to investigate whether TTV replicates in the liver and/or in peripheral blood mononuclear cells (PBMC) in patients with TTV infection and to compare the TTV strains present in 3 different compartments: liver-serum-PBMC.

Methods: TTV DNA was amplified with nested PCR and the concentrations in serum, liver and PBMC were assessed semi-quantitatively by end point dilutions of samples from 9 individuals with TTV infection. Direct DNA sequencing of amplicons from different compartments and phylogenetic analysis were performed to characterise the TTV isolates. The presence of TTV RNA, as a marker of virus replication, was sought by reverse transcription PCR (using TTV specific primers) of mRNA extracted and purified from liver specimens and PBMC.

Results: TTV DNA was found in all 3 compartments and the phylogenetic analysis revealed that different TTV genotypes were present in serum, PBMC and liver of the same patient. Despite this diversity in virus population in the same host, the TTV strains isolated from liver specimens of different cases showed close clustering in phylogenetic trees. Although the TTV DNA concentrations in liver and PBMC were 10 or more times higher than the serum levels in the same case, TTV RNA was detected only in liver and PBMC. This suggests a possible mechanism for the emergence of antigenically altered surface escape HBV variants, which are selected by high doses of HBIG after liver transplantation.

06 Evidence that TTV-virus is hepatotropic: detection of virus-specific transcription in liver specimens from patients with TTV infection

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It is currently unknown whether TTV production is restricted to the liver or spreading to other organs. We quantified TTV RNA in vivo in all 3 compartments of different cases of chronic TTV infection, using specific primers for TTV nucleocapsid and core genes. Furthermore, in one case, we also quantified the load of HBV DNA. We found that TTV RNA is highly variable among patients, and that the TTV load is lower in serum and PBMC than in liver. These results demonstrate for the first time that TTV is a hepatotropic virus, with virus-specific transcripts detectable in the liver, while TTV DNA sequenced in PBMC shows no evidence of replication.
08 Deficient expression of interleukin-12 receptor on T lymphocytes from patients with chronic HBV infection

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Interleukin-12 (IL-12) is a cytokine produced by antigen presenting cells which promotes cell-mediated immunity by inducing T helper 1 type responses and interferon-gamma production in lymphocytes. IL-12 binds to a Bl/B2 heterodimeric IL-12 receptor (IL-12R) on T lymphocytes and NK cells. IL-12R deficiency has been shown to result in impaired immunity to intracellular bacteria. The presence of weak T cell responses to hepatitis B virus (HBV) is the dominant cause of chronic HBV infection and in this study we tested the hypothesis that T lymphocytes from patients with chronic HBV hepatitis B have reduced IL-12R expression.

Methods: We studied 13 patients with chronic hepatitis B virus infection (seropositive for HBsAg and HBV DNA); 12 healthy controls and 7 of the HBsAg positive patients.

Results: IL-12R expression was found in a significantly lower proportion of T lymphocytes from patients with chronic HBV infection with a mean (SD) 5.4 (4.2)% compared with 10.2 (4.4)% (6.5)% p=0.0003, Mann-Whitney U and in comparison with patients with chronic hepatitis C (20.3 (16.7)%); p=0.02, Mann Whitney U. In vitro PBMC proliferative response to recombinant IL-12 (10 ng/ml) was determined by [3H]thymidine uptake. In addition, the in vivo effect of exogenous IL-12 on IL-12R expression was evaluated in 7 of the HBsAg positive patients.

Conclusions: Results demonstrate that T lymphocytes from patients with chronic HBV infection have reduced expression of IL-12R. The receptor remains functional and the expression is upregulated by exogenous IL-12 application.

09 Hyperammonemia following a simulated bleed results in neuropsychological deterioration and regional cerebral deactivation

R JALAN, H F LUI, S W M OLDE DAMINK, M GLARUS, k EMERSON, P C HAYES
Liver Unit, Royal Infirmary, Edinburgh, UK

Background: Ammonia is the most important factor in the pathogenesis of hepatic encephalopathy (HE) and diuretic induced dehydration is the most important precipitant of HE in cirrhosis. Inhibition of the angiotensin converting enzyme reduces renal ammonia excretion and angiotensin II stimulates ammonia production by the proximal tubules. The aims of this study were to test the hypothesis that plasma volume expansion in cirrhosis is followed by reduction in plasma ammonia concentrations that is mediated through neurohumoral mechanisms, particularly the renin-angiotensin system. Methods: Ten patients with previous TIPSS (mean age 52.2 (±3.1), M/F=6/4, mean Pugh - 7 (±3.1)) for variceal bleeding and previous ascites, and 6 pre-ascitic cirrhotics (mean age 54 (±2.1), M/F=4/6, mean Pugh=6.1 (±0.5)) with previous variceal bleed were studied. Venous ammonia concentration, glomerular filtration rate (GFR), renal plasma flow, urinary sodium excretion (UNaV), plasma renin activity (PRA), angiotensin II (AII), atrial natriuretic factor (ANF) and endothelin-1 (ET-1) were measured before and for 3 hours after an injection of 15% saline over 1 hour. Differences within the group was tested using ANOVA. Data are expressed as mean (±SEM). Renal and femoral fluxes are expressed per two kidneys/legs. PDV: portal drained viscera. Results: There was no change in arterial 15N-H2O in TIPSS patients. The peak difference between baseline and TIPSS was 41.1 (0.8), t=3hrs: 40.9 (0.3). Blood flow across the different organs did not change (table 1). Conclusion: Hyperammonemia after an (simulated) UGI bleed in patients with cirrhosis of the liver is mainly the result of enhanced renal ammonia production. During hyperammonemia, in patients with cirrhosis of the liver and TIPSS, muscle is the major site of ammonia detoxification. New therapeutic strategies that aim to diminish ammonia production after variceal bleeding in patients with cirrhosis should aim at renal ammonia production.

11 Modulation of hyperammonemia in cirrhosis by neurohumoral mechanisms

R JALAN, S W M OLDE DAMINK, P C HAYES
Liver Unit, Royal Infirmary, Edinburgh, UK

Background: Ammonia is the most important factor in the pathogenesis of hepatic encephalopathy (HE) and diuretic induced dehydration is the most important precipitant of HE in cirrhosis. Inhibition of the angiotensin converting enzyme reduces renal ammonia excretion and angiotensin II stimulates ammonia production by the proximal tubules. The aims of this study were to test the hypothesis that plasma volume expansion in cirrhosis is followed by reduction in plasma ammonia concentrations that is mediated through neurohumoral mechanisms, particularly the renin-angiotensin system. Methods: Ten patients with previous TIPSS (mean age 52.2 (±3.1), M/F=6/4, mean Pugh - 7 (±3.1)) for variceal bleeding and previous ascites, and 6 pre-ascitic cirrhotics (mean age 54 (±2.1), M/F=4/6, mean Pugh=6.1 (±0.5)) with previous variceal bleed were studied. Venous ammonia concentration, glomerular filtration rate (GFR), renal plasma flow, urinary sodium excretion (UNaV), plasma renin activity (PRA), angiotensin II (AII), atrial natriuretic factor (ANF) and endothelin-1 (ET-1) were measured before and for 3 hours after an injection of 15% saline over 1 hour. Differences within the group was tested using ANOVA. Data are expressed as mean (±SEM). Renal and femoral fluxes are expressed per two kidneys/legs. PDV: portal drained viscera. Results: There was no change in arterial 15N-H2O in TIPSS patients. The peak difference between baseline and TIPSS was 41.1 (0.8), t=3hrs: 40.9 (0.3). Blood flow across the different organs did not change (table 1). Conclusion: Hyperammonemia after an (simulated) UGI bleed in patients with cirrhosis of the liver is mainly the result of enhanced renal ammonia production. During hyperammonemia, in patients with cirrhosis of the liver and TIPSS, muscle is the major site of ammonia detoxification. New therapeutic strategies that aim to diminish ammonia production after variceal bleeding in patients with cirrhosis should aim at renal ammonia production.

Abstract 10, Table 1 V-A ammonia differences (µM) and ammonia fluxes (nmol/kg bw/min)

<table>
<thead>
<tr>
<th>Time</th>
<th>Art</th>
<th>PDV</th>
<th>Femoral</th>
<th>Kidney</th>
<th>Liver 100%</th>
<th>Liver 70%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>74 (7)</td>
<td>520 (183)</td>
<td>-483 (154)</td>
<td>67 (105)</td>
<td>-221 (93)</td>
<td>-267 (96)</td>
</tr>
<tr>
<td>1</td>
<td>109 (8)</td>
<td>975 (358)</td>
<td>-564 (80)</td>
<td>599 (150)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>122 (6)†</td>
<td>768 (243)</td>
<td>-825 (115)</td>
<td>575 (171)†</td>
<td>-180 (138)</td>
<td>-324 (109)</td>
</tr>
</tbody>
</table>

Statistics: † p<0.001; ‡ p<0.05. Negative fluxes represent uptake, positive: release.
using ANOVA with repeated measures. Relationship between variables was tested using linear regression. Results: No significant changes were observed in the mean arterial pressure or heart rate during the experiment. Results are summarised in the table below (table 1). No significant changes were observed in the concentrations of ET-1, cGMP and NA. The change in ANG correlated significantly with the change in GFR (r=0.6, p<0.01), urinary ammonia excretion (r=0.65, p<0.01) and with the change in plasma venous ammonia concentrations (r=0.7, p<0.01). Conclusion: Acute volume expansion reduces systemic ammonia concentrations significantly. Assuming a total volume of distribution of ammonia of 73% body weight, total body ammonia was reduced by 0.95 mmol/hr. The increase in renal ammonia excretion was 0.5 mmol/hr suggesting that renal ammoniagenesis was reduced, possibly mediated by reduced Angiotensin II. The results of this study highlight an alternative target for reducing ammonia and treating HE.

12 Changes in renal blood flow, systemic haemodynamics and hepatic blood flow following acute increase in portal pressure is mediated by regional endothelin production

R JALAN, N REDHEAD, P C HAYES
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Background and Aims: Acute increase in portal pressure following acute occlusion of a transjugular intrahepatic stent-shunt (TIPS) produces significant reduction in renal blood flow (RBF) and increased systemic vascular resistance (SVR) (Jalan et al. Gut 1997;40:664–70). This study was designed to test the hypothesis that these changes are mediated by local production of endothelin (ET). Methods: Following routine portography, the shunt was acutely occluded with a balloon for 12 min in 16 patients (age=54.2±5.2, M/F-10/6, Pugh 8.1). These patients were entirely stable and on no diuretic therapy for at least 1 week prior to the procedure. Hepatic blood flow (HBF) was measured in all patients using indocyanine green clearance; in 8 patients RBF was measured with a reverse thermodilution catheter placed in the renal vein; cardiac output and SVR were measured using a Swan Ganz catheter in the other 8 patients. Measurements were made before, during (6 and 12 min) after shunt occlusion. Blood was collected from an artery, hepatic and renal veins at the above time points. Data were expressed as mean (SEM) and changes within a group were measured using ANOVA with repeated measures. Results: No significant changes were observed in the heart rate or the mean arterial pressure. Results of haemodynamic measurements are summarised in the table below (table 1). Systemic and renal vein ET-1 and Big ET-1 increased significantly (systemic=−3.1±0.3), to 5.8±0.6 pg/ml, p<0.01 and 33.4±3.3 to 39.1±3.1 pg/ml, p<0.05; renal=−3.3±0.4 to 9.8±0.9 pg/ml, p<0.001, and 37±4.1 to 55±4.5 pg/ml, p<0.01; hepatic=−3.6±0.5 to 2.9±0.6 pg/ml, p<0.05 and 35±3.1 to 27±2.1 pg/ml, p<0.05). The change in SVR, RBF and HBF correlated with the changes in Big ET-1 concentrations (r=0.7, p<0.05 and r=0.5 respectively). Conclusions: The results of this study show that the reduction in RBF and increase in HBF and SVR following acute increase in portal pressure is mediated by regional endothelin production. This study provides the haemodynamic rationale for the use of selective endothelin antagonists in the complications of portal hypertension.

13 Inhibition of nitric oxide synthase improves liver damage and reduces mortality in a characterised model of thioacetamide induced acute hepatic failure

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Introduction: The role of nitric oxide (NO) in the pathogenesis of acute hepatic failure (AHF) is controversial. Studies investigating inducible nitric oxide synthase (iNOS) examining parameters of AHF and mortality have shown conflicting results. Aims: To investigate the role of iNOS inhibition in the pathophysiology of a fully characterised, reproducible model of AHF. Methods: We have developed a robust, reproducible thioacetamide (TAA) induced model of AHF in the Wistar rat. This has been extensively characterised in terms of clinical sequelae, biochemical, metabolic and haematological parameters over 96 hours post induction. AHF was induced in Wistar rats by two intraperitoneal (IP) injections of 500mg/kg b.w. Three groups of animals were each studied at three time points (24,48,72 hours. n=5). Group 1 received TAA only. Group 2 and 3 also received TAA, however, Group 2 were pre-treated with the NO precursor L-Arginine (300 mg/kg b.w. IP) once daily for seven days and Group 3 were pre-treated with NO synthase inhibitor aminoguanidine (100mg/kg sub-cutaneously) for three days. Survival was separately assessed for each group with animals being left for 96 hours. Results: (n+/−SEM) *P<0.05 **P<0.005 (table 1). Survival: At 96 hours survival in Group 1 (TAA) was 35% (n=10), Group 2 was 10% (n=10) and Group 3 (n=5). Conclusion: Our results using a validated reproducible rat model of AHF indicate that iNOS inhibition with aminoguanidine improves survival dramatically and also reduces all parameters of clinical, biochemical and histological hepatic damage following thioacetamide induced hepatotoxicity. Contrarily, supplementation with an NO donor led to increasing evidence of hepatic damage and lethality.

14 CD40 activation induced Fas (CD95) mediated apoptosis as a mechanism for intrahepatic bile duct loss

S C AFFORD, S RANDHAWA, J YOOSTER, L S YOUNG, D H ADAMS
The Liver Research Laboratories/MRC Centre for Immune Regulation, The University of Birmingham Institute of Clinical Science, Queen Elizabeth Hospital, and The CRC Institute for Cancer Studies, University of Birmingham Medical School, Birmingham, UK

Background: Bile duct loss is a characteristic feature of several liver diseases, including Primary Biliary Cirrhosis (PBC). The mechanisms underlying bile duct loss are poorly understood, although Fas (CD95) mediated apoptosis may be involved. We have reported recently that activation of another TNF receptor superfamily member, CD40, can amplify hepatocyte apoptosis by triggering autocrine activation of Fas. Based on this work we hypothesised that CD40 might be a crucial factor in regulating Fas-mediated apoptosis of biliary epithelial cells (BEC). Methods: CD40 and Fas expression was assessed using immunohistochemistry in tissues from patients with end stage PBC. BEC were isolated from liver tissue and cultured in the presence or absence of IL-1, TNFα, IFNγ and TGFβ. CD40, CD40L, Fas and FasL protein and mRNA expression were measured under these conditions.

Abstract 11, Table 1

<table>
<thead>
<tr>
<th>Pre saline infusion</th>
<th>2 hours after saline infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonia (µmol/l)</td>
<td>93 (±6.9)</td>
</tr>
<tr>
<td>GFR (ml/min)</td>
<td>154 (±22)</td>
</tr>
<tr>
<td>ERPF (ml/min)</td>
<td>682 (±136)</td>
</tr>
<tr>
<td>UNA (µmol/min)</td>
<td>143 (±18)</td>
</tr>
<tr>
<td>PRA (ng/ml/hr)</td>
<td>3.2 (±0.8)</td>
</tr>
<tr>
<td>Angiotensin II (pg/ml)</td>
<td>7 (±2)</td>
</tr>
<tr>
<td>ANP (pg/ml)</td>
<td>4.6 (±1.1)</td>
</tr>
</tbody>
</table>

‡p<0.001, †p<0.05.

Abstract 12, Table 1

<table>
<thead>
<tr>
<th>Pre-occlusion</th>
<th>12 min after occlusion</th>
<th>Following deflation</th>
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</thead>
<tbody>
<tr>
<td>RBF (ml/min)</td>
<td>296 (±40)</td>
<td>139 (±270)†</td>
</tr>
<tr>
<td>Cardiac output (l/min)</td>
<td>7.4 (±0.5)</td>
<td>136 (±139)‡</td>
</tr>
<tr>
<td>SVR (dyn.sec/cm5)</td>
<td>11.31 (±93)</td>
<td>51.2 (±22.1)‡</td>
</tr>
<tr>
<td>Portal pressure (mmHg)</td>
<td>7.6 (±0.4)</td>
<td>898 (±372)</td>
</tr>
<tr>
<td>HBF (ml/min)</td>
<td>745 (±23)</td>
<td>789 (±226)</td>
</tr>
</tbody>
</table>

†p<0.001, ‡p<0.05.

Abstract 13, Table 1

<table>
<thead>
<tr>
<th></th>
<th>AST (iu/l)</th>
<th>NH₃ (µg/ml)</th>
<th>INR</th>
</tr>
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<tbody>
<tr>
<td>24</td>
<td>48</td>
<td>24</td>
<td>24</td>
</tr>
<tr>
<td>TAA</td>
<td>165±3/164</td>
<td>403±5±247</td>
<td>54±9</td>
</tr>
<tr>
<td>TAA+L-ARG</td>
<td>2177±2/231</td>
<td>4475±2/240</td>
<td>126±3*</td>
</tr>
<tr>
<td>TAA+AMG</td>
<td>346±5*</td>
<td>1090±186**</td>
<td>49±5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Lactate (mMol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>48</td>
</tr>
<tr>
<td>TAA</td>
<td>5.54±0.7</td>
</tr>
<tr>
<td>TAA+L-ARG</td>
<td>7.2±0.2</td>
</tr>
<tr>
<td>TAA+AMG</td>
<td>3.5±0.2*</td>
</tr>
</tbody>
</table>

AMG = Aminoguanidine; INR = International Normalised Ratio; TAA = Thioacetamide
were simultaneously assessed by flow cytometry and RT PCR over 48hrs. Apoptosis was assessed by in situ DNA end labelling and morphometry, following activation of BECs with crosslinking antibodies to CD40 or Fas. Results: CD40 and Fas were found to be strongly co-expressed in perisclerotic ducts in PBC. In vitro, BEC expressed CD40, Fas and Fasl, but not CD40L. CD40 expression was upregulated by TNF-α whereas other cytokines had no effect. Activation of CD40 was a potent stimulus for BEC apoptosis (comparable with direct activation of Fas) and was associated with a 2–3 fold increase in steady state levels of FasL mRNA. CD40 induced apoptosis was prevented by a neutralising anti-FasL antibody (NOK 1). Summary: 1) BEC can express and synthesise CD40, Fas and Fasl, but not CD40L. 2) BEC CD40 expression is inducible by proinflammatory cytokines and 3) Activation of CD40 on BEC leads to increased FasL, expression and Fas-mediated apoptosis. Conclusions: These data suggest that CD40 activation of Fas-mediated apoptosis is an important mechanism in determining BEC apoptosis in inflammatory liver disease.

15 Interferon-alpha 2b/ribavirin combination therapy for chronic hepatitis C infection: a single centre experience

P L SHIELDS1, D MUR1, T WILDE2, D J MUTIMER1,2 'Liver and Haemophilia Units, Queen Elizabeth Hospital, Edgbaston, Birmingham, UK Interferon (IFN) therapy used at the Liver Unit between 1991–97 in 57 patients (pts) chronically infected with hepatitis C virus (HCV) has achieved sustained viral clearance of less than 20%. Promising early results of the ribavirin/IFN combination prompted an open label, double-blind, randomised, placebo controlled trial in 148 pts investigating efficacy and safety of a 1 year course of ribavirin (1–1.5 g daily) in combination with IFN alpha 2b (3MU TWS weekly). Ninety eight HCV Riba +ve, serum HCV RNA+ve pts including 28 with hereditary bleeding disorders have undergone therapy since 1997. The majority were non cirrhotic (median: HAI 6/13 with mild/moderate fibrosis). Virological responses (determined by loss of serum HCV RNA) at 3 and 6 months of therapy were: 62% (92/148) and 71% (105/148) respectively. Side effects during therapy were commonly seen and included leucopenia associated with ribavirin induced haemolytic anaemia, generalised fatigue, skin rash, pruritus, anaemia and thyroid dysfunction. In 14 of 98 (14%) pts severe side effects required early cessation of therapy after 1–11 (m)onths; 9/14 of these have had a sustained viral response. Atypical responses to therapy with persistently elevated ALT activities despite loss of HCV RNA were seen in 4 pts; in 2 of these ALT values normalised after discontinuation of therapy suggesting that their transaminases might be autoantigen induced. 39 of 70 (56%) pts (including the above 14 who stopped early) that completed IFN/Ribavirin had a durable biochemical (ALT) and virological response with a median follow up of 7(range 1–24) m. Treatment was ongoing in 28 pts. Combination therapy with IFN alpha 2b and Ribavirin represents a significant therapeutic advance in chronic HCV infection.

16 The MHG class II molecule HLA-DQB1*0301 is a marker of hepatitis C infection, but is associated with mild chronic hepatitis or viral clearance

C L JACKSON, F M THOMPSON, N TUNER, W M HOWELL, W M ROSENBERG Cell and Molecular Medicine, Southampton General Hospital, Southampton, UK Introduction: Hepatitis C virus causes a chronic hepatitis in over 80% of those infected. The virus is not cytopathic and the associated liver pathology is immune mediated and varies in its severity between infected individuals. Male sex, increasing age and high alcohol intake are recognised as poor prognostic indicators, and it is likely that immune and genetic factors are also involved. HLA-DQB1*0301 has been shown to be over-represented in patients who have spontaneously cleared the virus. We tested the hypothesis that HLA-DQB1*0301 is protective from disease progression in chronic hepatitis C (CHC). Methods: We identified two cohorts of patients, with either mild or severe CHC on liver biopsy and matched for known disease modifying factors. Genomic DNA was extracted from citrated whole blood using a proteinase K Digestion and rapid salt out technique. HLA-DQB1 genotyping was established using allele specific primers in ARMS-PCR amplification. PCR products were resolved on 3% agarose gels, digested and allele specific fragments separated by agarose electrophoresis. We compared the frequency of HLA-DQB1*0301 between the CHC cohorts and the background population, and between the two cohorts of CHC patients who were discordant disease diagnosis (mild CHC). Results: The frequency of HLA-DQB1*0301 in the background population was 33% (99/300), which is consistent with the findings of other groups. The frequency in all patients with CHC was 40% (1230). In mild disease the allele occurred in 64% (7/11), whilst in severe disease the frequency was 26% (5/19), (p=0.05). Conclusions: HLA-DQB1*0301 is found more frequently in HCV antibody positive individuals than in the general population. This allele is over-represented in patients with mild CHC and less frequent in more severe hepatitis. These data suggest that the HLA-DQB1*0301 is a marker of HCV infection and mild CHC, although the mechanism is not clear. The observed association may be explained by allele specific differences in host cell responses to infection, antigen presentation, or due to linkage with other genetic loci such as TNF-a. In conjunction with previous data suggesting that HLA-DQB1*0301 is associated with acute resolved HCV infection, this allele may be considered to be a marker of good prognosis.

17 Antigen specific CD4+ T lymphocyte proliferative responses to hepatitis C non-structural NS3 region differentiates patients with mild and severe hepatitis

F M THOMPSON, C L JACKSON, W M ROSENBERG Cell and Molecular Medicine, Southampton General Hospital, Southampton, UK Introduction: Hepatitis C virus (HCV) causes a chronic hepatitis in over 80% of infected individuals. The liver injury associated with chronic hepatitis C (CHC) consists of periportal lymphocytic infiltrates with hepatocytic necrosis and a variable degree of progressive fibrosis. In chronic infection, the disease can establish one of three patterns; 1/3 mild liver disease, 1/3 moderate disease with some fibrosis, and 1/3 progressive fibrosis leading to cirrhosis. Disease progression is complicated by hepatocellular carcinoma. We have investigated the correlation between HCV antigen specific CD4+ T lymphocyte responses and disease severity, in patients with chronic hepatitis C. Methods: We selected two HCV peptides for their promiscuity in class II HLA binding and conservation across viral genotypes. (Core: amino acids (aa) 23–42, NS3: aa1248–1261). Tatanus toxoid (aa947–967) was used as a control peptide. Two cohorts of patients were identified, differing in severity of CHC on liver biopsy, matched for known disease modifying factors (n=14 in each group). Peripheral blood mononuclear cells were separated by density centrifugation and plated for culture at a density of 1x10⁶ cells/L. Cells were cultured for 4–6 days in the presence or absence of 10µg/ml peptide. 0.5µCi H’timidine was added to each well for the final 16 hours. The cells were washed and assayed for proliferation by measuring inorganic phosphate release. Results: The mean PI for each peptide was not significantly different between the CHC positive cohort compared to controls. The number of patients with a PI above the level of significance for NS3, was 28% in patients with severe disease and only 7% in those with milder disease. (Range of PI in controls = 0.79–1.32). Conclusions: There are differences in the peptide specific CD4+ T cell responses of patients with differing severity of CHC. Proliferative responses to peptides are likely to be less frequent and more subtle than those seen with whole proteins. The higher frequency of responders seen in severe disease may reflect more vigorous antigen specific immune responses, contributing to more aggressive liver damage. The finding of immune reactivity to NS3 is consistent with other studies.

18 Exogenous and endogenous interleukin-10 modulates various aspects of hepatic stellate cell function

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Interleukin-10 (IL-10) is an anti-inflammatory cytokine which downregulates most of the pro-inflammatory actions of macrophages. In addition, IL-10 has been shown to modulate matrix metalloproteinases (MMP) and inhibits metalloproteinase-1 (TIMP-1) expression in monocytes and to reduce collagen synthesis in fibroblasts. Hepatic stellate cells (HSC) synthesise IL-10 during activation in vitro. In a carbon tetrachloride induced model of liver fibrosis, IL-10 deleted (IL-10ΔT) mice developed significantly more fibrosis than matched wild type controls despite similar levels of inflammation. These data suggest that IL-10 may downregulate liver fibrosis both indirectly via macrophage inactivation and directly via effects on HSC. We have examined expression of the IL-10 receptor on HSC and the effects of exogenous recombinant IL-10 and neutralising anti-IL-10 antibody on various aspects of HSC function including proliferation, pro-collagen-1, alpha smooth muscle cell actin (α-SMA), gelatinase-A and TIMP-1 mRNA synthesis in vitro. Rat HSC were cultured for up to 14 days in the presence or absence of IL-10 (50 ng/ml), anti IL-10 (2µg/ml) or appropriate control media. IL-10 receptor mRNA expression was assayed by RTPCR in passages mouse HSC with or without 24 hours pre-stimulation with LPS.
Neither rIL-10 nor anti-IL-10 antibody had any effect on HSC proliferation as assessed by 3H-thymidine incorporation. Levels of other mRNAs were assessed by northern hybridisation and normalised to β-actin levels. Pro-collagen-I mRNA was reduced to 55% of control by rIL-10 (p<0.002) and increased to 144% by anti-IL-10 (p<0.05). Gelatinase-A mRNA was reduced to 80% (ns) by rIL-10 and increased to 114% by anti-IL-10 (p<0.02). Levels of c-SMA mRNA were unchanged by rIL-10 but increased to 148% by antibody (p<0.05). TIMP-1 mRNA levels were reduced to 63% of control by rIL-10 (p<0.01) and marginally increased by anti-IL-10 (ns). These data suggest that both rIL-10 and endogenous IL-10 can directly modulate HSC function with a net potentially anti-fibrotic effect.

19 Expression of the hematopoietic markers CD34 and c-het in human liver explant cultures. H A CROSBY, V Hexter, A J STRAIN

1 Dept. of Biochemistry, Pathology, University of Birmingham & Liver Unit, Birmingham Children’s Hospital, Birmingham, UK

In rodents, following severe liver injury, oval cells have the capacity to grow and differentiate into hepatocytes or biliary epithelial cells and are thought to arise from a stem cell population of progenitor cells that reside at the border between hepatocytes and non-parenchymal cells in an embryonic mosaic pattern. In cirrhosis, hepatocyte progenitor cells are hypothesised to give rise to hepatocyte cells. Sections from primary human liver biopsies (n=5), Alcoholic Liver Disease (n=5) and Biliary Atresia (n=5) were immunostained using CD34 and c-het antibodies. A panel of markers recognising hepatocytes, biliary epithelium and non-parenchymal cells was used to characterise these cells. Additionally, cells were immunonuclideated using CD34 and c-het antibodies from collagenase digestion of normal and diseased human liver, were cultured using growth media and the cell phenotype examined by electron microscopy (EM). Culture of hepatocyte-alone demonstrated that albumin synthesis which peaked at day 3, remained steady throughout the run (approximately 500ng/hr/10^5 cells) indicating good cell survival and function. Hepatocytes also synthesised urea and MegX at rates which compared favourably with in vitro studies and EM showed good attachment on the capillary surfaces. The addition of NAC resulted in a similar trend to single culture overall, however, albumin output was substantially higher (approximately 10 fold) compared to uptake of 500ng/hr/10^5 cells. MegX and urea synthesis was also markedly improved in the co-culture system with peaks on average of x5 & x3 fold higher respectively. The benefits of hepatocyte-NPC cell interactions was confirmed by EM showing preservation of three dimensional structure and the production of extracellular matrix. In conclusion these findings demonstrate that hepatocyte-NPC cell interactions is fundamental to ensure normalisation of hepatic endothelium and a flow-based adhesion assay both that could be blocked by 10G3 or ACT-1. Summary: Functionally active MADCAM-1 is expressed on liver endothelium in a majority of patients with PSC and AAI. Large numbers of u47 lymphocytes are detected in the circulation of patients with IBD and PSC and these cells bind to MADCAM-1 in vitro. Gut derived memory T cells traffic to liver endothelium in PSC/AIH. Conclusions: The recruitment of u47 high mucosal lymphocytes to the liver is promoted by binding to MADCAM-1 on liver endothelium thereby providing a mechanism to modulate hepatic inflammation in patients with IBD.


1 On behalf of Trent HCV Study Group
Department of Infectious Diseases, Gastroenterology, and Pathology, Royal Hallamshire Hospital Sheffield, Department of Public Health, Microbiology and Gastroenterology, Nottingham, National Blood Authority, Sheffield, UK

Background: The epidemiology of hepatitis C in the United Kingdom is incompletely understood. The Trent HCV study is a five city group based in South Yorkshire and East Midlands of the UK and was initiated in 1991. Aim: To study the epidemiology, natural history and progression of HCV infection in this community based geographical cohort. Methods: Patients presenting to 15 centres with HCV antibody were referred to one of 5 centres. Patients are investigated and treated according to an agreed common protocol and an epidemiological database has been established. Results: Between 1991 and
was assessed as adequately nourished (AN), appropriately referenced (MAMC) was determined and independently associated with HGS (p<0.005) (table 1). Conclusion: Measurement of hand-grip strength provides a simple and quick method of assessing nutritional status in patients with cirrhosis which is independent of the degree of hepatic decapitation.

24 Gliotoxin promotes hepatic stellate cell apoptosis via a caspase dependent mechanism

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1University Medicine, University Surgical Unit, Southampton General Hospital, Southampton, UK

In response to liver damage, stellate cells within the liver “activate” to a myofibroblast-like (α-smooth muscle positive) phenotype that is responsible for the majority of extracellular matrix protein deposition that characterises liver fibrosis. In rats, recovery from liver fibrosis—after termination of a toxic insult—is associated with a significant increase in stellate cell number (Jredale et al. J Clin Inv 1998;102,538–49). A potential target for the treatment of liver fibrosis may therefore be stellate cells and a stimulation in their rate of apoptosis. We have previously shown that the fungal toxin gliotoxin inhibited the constitutive NFκB DNA binding activity present in freshly isolated rat hepatic stellate cells and stimulated the cells to undergo apoptosis. The effect of gliotoxin on culture-activated rat and human hepatic stellate cells has therefore been examined. Gliotoxin added to the medium of culture activated rat and human liver stellate cells at concentrations greater than 300nM resulted in morphological alterations within 1 hour such as blebbing, rounding up and detachment from the sub-stratum. Within 2 hours, a nuclosomal ladder characteristic of apopto- sis was detectable in DNA isolated from rat stellate cells but not human stellate cells. Addition of the thiol reducing agents dithiotherol or PDTC to gliotoxin-treated rat cells blocked both morphological changes and DNA cleavage suggesting that the disulphide bridge present in gliotoxin is essential for the stimulation of apoptosis. In contrast, addition of the protease inhibitors Z-VAD-FMK (an irreversible inhibitor of caspases) and N-tosyl-L-phenyl chloromethyl ketone (a serine protease inhibitor) to gliotoxin-treated rat cells did not prevent morphological alterations but did block DNA cleavage. These observations sug- gested that DNA cleavage is downstream of the morphological changes induced by gliotoxin and require the functional activity of caspases and serine proteases activity. These data suggest that gliotoxin could be used to modu- late liver stellate cell numbers in the liver and the effects of gliotoxin on in vitro model of liver fibrosis is presently under investigation. This work is funded by a grant from the Wellcome Trust.

25 Distinct patterns of chemokine secretion and chemokine receptor expression shape the inflammatory response in rejecting human liver transplants

S GODDARD, A WILLIAMS, S G HUBSCHER, D H ADAMS
The Liver Research Laboratories, MRC Centre for Immune Regulation, University of Birmingham, UK

Background: Graft rejection after liver transplantation is associated with a lymphocytic infiltrate, the nature of which will be determined by the local activity of chemotactic cytokines (chemokines) that attract particular subsets of effector cells to the liver. The liver is a source of chemokines during rejection but little is known about the chemokine receptors used by lymphocytes infiltrating the liver. In order to determine which chemokine/chemokine receptor interactions are important in liver allograft rejection we determined: expression of chemokines and chemokine receptors in human liver grafts by immunohistochemistry and on circulating lymphocytes by flow cytometry; the relative contributions of these receptors to the effect of in vitro activation of chemokine receptor expression on lymphocytes. Results: Normal human liver and liver biopsies from patients with stable transplants contained a light infiltrate of lymphocytes and macrophages. Mortality was significantly lower in transplant recipients on active detailed questioning who had score of 6 or greater, 36(11%) patients were cirrhotic at presentation. There was no significant difference in Knodell score between patients who acquired hepatitis C through transfusion compared with IDU. Main genotype was 1, however, genotype 3a was proportionally more common in drug users. 153 patients were treated with interferon; response rate (PCR negative 6/12 post cessation of therapy) was 7.5%. 9 patients developed hepatocellular carcinoma. Data on alcohol consumption were available on 331 patients, which showed that higher consump- tion correlated with increased fibrosis on liver biopsy.

Aim: To measure hand-grip strength (HGS) in cirrhotic patients and to observe its influence being the most important cause of death. The main risk for acquisition is IDU, with very limited number of patients having no identifiable risk factor (lowest reported). Higher alcohol consump- tion and evidence of previous hepatitis B-infection correlated with a worse fibrosis score. Only genotype 3a correlated with the more advanced fibrosis. Mortality was surpris- ingly high for a young cohort with hepatic dis- ease being the most important cause of death.

23 Hand-grip strength in cirrhosis: its relationship to nutritional status and the degree of hepatic decapitation

A M MAXDEN, M Y MORGAN
Department of Medicine, Royal Free and University College Medical School, London, UK

Background: The evaluation of muscle strength is the principal measure of nutritional status although the confounding effects of the severity of illness have not been assessed in cirrhotic patients. Aim: To measure hand-grip strength (HGS) in cirrhotic patients and to observe its relationship with anthropometric measure- ments, global nutritional status and the degree of hepatic decapitation. Methods: Consecutive patients with cirrhosis with no evidence of neurological or muscular disease (n=174; 99:75: mean [range] age, 49.4 [26–76] yr; Child’s grade, A 52%; B 28%; C 40%) were evaluated: (a) HGS was measured using the dominant arm with a Takley Instrument (Tokyo, Japan) and compared with reference data (JPNEN 1989;13:30); (ii) mid-arm muscle circumfer- ence (MAMC) was determined and appro- priately referenced (J Clin Nutr 1981;34:2530); (iii) global nutritional status was assessed as adequately nourished (AN), moderately malnourished (MM) or severely malnourished (SM) using a reproducible, validated method (J Hepatol 1997;26:61:125)

Results: Relative HGS was significantly associated with relative MAMC (r=0.28, p<0.0005); median HGS values were signifi- cantly lower in malnourished patients (p<0.0001) and in those with more severely decompen- sated liver disease (p<0.0005). However, only nutritional status was indepen- dently associated with HGS (p<0.005) (table 1). Conclusion: Measurement of hand-grip strength provides a simple and quick method of assessing nutritional status in patients with cirrhosis which is independent of the degree of hepatic decapitation.

Abstract 23, Table 1

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<td>A2</td>
<td>B3</td>
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Median (range) HGS%

N=72 (28–124) MM=57 (17–109) SM=54 (31–96)

December 1998, 1139 patients have been recruited to this cohort (775 male/364 female). 93% are Caucasians with median age 43 years (range 14–89). Risk factors included intravenous drug use in 73%, trans- fusion in 14%; 4.3% of patients had no identi- fiable risk factor on active detailed question- ing. PCR (Roche Amplipoc) was positive in 695/860 (81%); 553 liver biopsies have been conducted, of which 331 thus far Knodell score was recorded. 115 (35%) had score of 6 or greater, 36(11%) patients were cirrhotic at presentation. There was no significant difference in Knodell score between patients who acquired hepatitis C through transfusion compared with IDU. Main genotype was 1, however, genotype 3a was proportionally more common in drug users. 153 patients were treated with interferon; response rate (PCR negative 6/12 post cessation of therapy) was 7.5%. 9 patients developed hepatocellular carcinoma. Data on alcohol consumption were available on 331 patients, which showed that higher consump- tion correlated with increased fibrosis on liver biopsy. A similar observation was recorded in 185 patients who had previous evidence of hepatitis B co-infection. 55 deaths were recorded, 18 due to liver disease, 10 lifestyle (suicide/murder), 4 medical related, 12 unre- lated (no data). Condition: Hepatitis C in Trent Region is particularly common among young men. The main risk for acquisition is IDU, with very limited number of patients having no identifiable risk factor (lowest reported). Higher alcohol consump- tion and evidence of previous hepatitis B-infection correlated with a worse fibrosis score. Only genotype 3a correlated with the more advanced fibrosis. Mortality was surpris- ingly high for a young cohort with hepatic dis- ease being the most important cause of death.

23 Hand-grip strength in cirrhosis: its relationship to nutritional status and the degree of hepatic decapitation

A M MAXDEN, M Y MORGAN
Department of Medicine, Royal Free and University College Medical School, London, UK

Background: The evaluation of muscle strength is the principal measure of nutritional status although the confounding effects of the severity of illness have not been assessed in cirrhotic patients. Aim: To measure hand-grip strength (HGS) in cirrhotic patients and to observe its relationship with anthropometric measure- ments, global nutritional status and the degree of hepatic decapitation. Methods: Consecutive patients with cirrhosis with no evidence of neurological or muscular disease (n=174; 99:75: mean [range] age, 49.4 [26–76] yr; Child’s grade, A 52%; B 28%; C 40%) were evaluated: (a) HGS was measured using the dominant arm with a Takley Instrument (Tokyo, Japan) and compared with reference data (JPNEN 1989;13:30); (ii) mid-arm muscle circumfer- ence (MAMC) was determined and appro- priately referenced (J Clin Nutr 1981;34:2530); (iii) global nutritional status was assessed as adequately nourished (AN), moderately malnourished (MM) or severely malnourished (SM) using a reproducible, validated method (J Hepatol 1997;26:61:125)

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Median (range) HGS%

N=72 (28–124) MM=57 (17–109) SM=54 (31–96)
The patients were divided in two groups (Group1 (n=9), score. 7 required liver trans-plantation; Group2 (n=43) are alive and well on chelation therapy. The presenting fea-tures in 39 (Gr1/Gr2; p>0.001; t test) varied in 11 (6/5; p<0.01). The following features were not statistically different in the two groups (Gr1/Gr2); mean age 12–10 years, oesophageal varices 1/1, abdominal pain 4/6, Kayser–Fleischer rings 6/21, anaemia and lea-thery 6/9. The serum biochemistry and haematology was as follows: mean INR (inter-national normalised ratio) 2.9/1.3, p<0.0001; total bilirubin 409/20 µmol/l; AST 239/108 IU/L, p<0.001; alkaline phosphatase 148/324 IU/L, p=ns; albumin 27.3/7 µg/L, p<0.01; serum copper 17.5/5.5 µmol/l, p<0.001; haemoglobin 9.8/12.1 g/dL, p=ns. A receiver operator curve (ROC) was used to achieve highest area under the curve to pre-dict the prognostic value of the above men-tioned laboratory parameters. A combina-tion of INR >1.5, total bilirubin >100 µmol/l and serum copper >12 µmol/l had 100% sensitiv-ity, 97% specificity and 89% positive predictive value, in predicting the outcome of WD at presentation. The triad of INR >1.5, total serum bilirubin of >100 µmol/l and serum copper of >12 µmol/l at diagnosis appears to be a good prognostic marker as to the outcome of WD in children and may help in identifying the patients where liver trans-plantation should be considered.

28 Neonatal haemochromatosis in eleven families: pattern of presentation and outcome
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2Neonatal haemochromatosis (NH) is an un-common disorder in foetal life. Jaundice is the most noted onset is associated with massive iron depo-sition sparing the reticular-endothelial system. It seems to be related to maternal factors but the pattern of inheritance is unknown. Prognosis is poor. The role of antioxidant and iron chelation therapy is controversial. To review clinical features, treatment and outcome of NH, we analysed retrospectively the medical records of 15 children (6 males) from 11 fami-lyies referred to King’s College Hospital between 1990–1998. Diagnosis was based on clinical findings, high ferri-tin, iron deposition in the liver and other tissues confirmed by MRI, liver biopsy and/or post-mortem examination. In 8 of the 11 families, the affected child was the unique unaffected first child. Six families had further children and in 5 the disease recurred. In one family, 2 children from different fathers were affected. The sibling of affected child had normal non-specific liver function tests. There were 4 mis-carriages in 3 families. Oligohydramnios was observed in 4 of the 15 children, oedematous placenta in 4 and reduced foetal movements in 3. Four were born prematurely, including 1 who were induced who; one with a positive family history, had a pre-natal diagnosis of NH suggested by cordocentesis showing abnormal liver function tests and clothing at 30 weeks of gestation and the other was the third affected of a triplet non-identical gestation. Two children were below the 3rd percentile, 2 were at the 3rd and 10 were at or above the 10th percentile. Eleven presented with symptoms of hypoglycaemia and 4 in the first week (3 with jaundice and 1 with lethargy). Bleeding diathesis occurred in 6, ascites in 3, generalised oedema in 3 and splenomegaly in 1. Ferritin level was elevated in all patients (median: 19706/dL, range: 202–100000). Median AST was 108/15 (range: 28–1275) and median bilirubin 200 µmol/l (range: 55–759). Hypoalbuminaemia (median: 20g/l, range: 14–30) and raised INR (median: 3.2; range: 2.1–3) were constant in all. Extragastro-intestinal siderosis was demonstrated by MRI in 2 (pancreas) and by post-mortem examination in 9 (pancreas in 6, myocardium in 5, kidney in 4, thyroid gland in 4, adrenal glands in 3 and salivary glands in 2). Liver histology, available in 12, showed parenchymal collapse, diffuse fibrosis, giant cell transformation, regenerative nodules, cholestasis and heavy haemosiderin deposition. Six patients received a chelation-antioxidant cocktail (Fehat Res 1993; 33:109A), 3 in the first day of life and 3 between week 2 and 3. Two died at 35 and 92 days and the other 4 required liver transplan-tation (median age: 1 year, range: 7–39) of whom 2 died. One of 9 infants who did not receive the cocktail survived, 3 died and 5 required transplantation (median age: 18 days; range: 15–42). Three of 5 transplanted died (median age: 28 days; range: 14–56). Overall 10 died and 5 (4 transplanted) are alive after a median follow up of 24 months (range: 12–52 months, with no recurrence yet). The conclusion NH is a disease starting prenatally and presenting at birth with end stage liver disease and diffuse iron overload. A first unaf-fected sibling is frequent but the risk of recur-rence in following pregnancies is high. NH has a very poor prognosis. Chelation/antioxidant treatment is not effective in severe cases and liver transplantation is the only curative option.

29 Biliary atresia—from 95% mortality to 96% survival
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Twenty years ago, biliary atresia (BA) was untreatable with 2 year mortality of 95%. Kasaiporto-enterostomy (KPE) and liver transplantation (OLT) have radically improved the outcome but their relative roles have not been clear. At our institute a multi-disciplinary team including paediatricians, paedi-astric and transplant surgeons provides con-tinuous care for liver disease. The aim was to document the results of this approach to BA and to describe an outcome classification of BA to permit consistent reporting of BA out-come. Between 1/3/93 and 28/2/95, 50 infants were referred to KCH with BA. KPE was performed in all at mean age 4 (14–120) days. At median 56 (range 47–73) months, 2 were referred for OLT elsewhere and are not considered in the analysis, I has been lost to follow-up at 17 months, 7 are anicteric (serum bilirubin <20 µmol/l), 20 with normal asparto amine-transferase and glutamyl transpeptidase (LFT) and no portal hypertension (PHT), 6 are anicteric with abnormal LFT but no PHT/ultrasound evidence only of splenomegaly, 1 anicteric with clinical evidence of PHT, 6 also have significant PHT of whom 3 have impaired nutrition, 2 have serum bilirubin >200 µmol/l, and are also listed for OLT, 10 have undergone OLT successfully all except 2 with normal LFT. One child died after OLT and 1 while listed. Thus KPE has 64% 5 year anicteric survival rate and overall actuarial 5 year survival is 96%. These data are better than any others published for BA, illustrating the benefits of an integrated service including low KPE failure rate with reduced need for OLT and less competition for organs, better management of chronic liver disease and a better recognition of need for OLT, excellent OLT outcome. We believe this model is the optimum for management of BA.
in this setting is limited. We describe our initial experience in nine paediatric patients (6 male, mean age 9 years, range 2.3–16) with acute liver failure (cryptogenic 6, autoimmune 1, mushroom poisoning 1, paracetamol overdose 1) who fulfilled criteria for emergency transplantation. Preoperatively, the mean international normalised ratio (INR) was 4.7 (range 2.8–13), with a serum bilirubin of 396 µmol/l (34–585). All patients received a compatible left (in 3) or right (4) auxiliary graft. Average time from listing to transplanting was 1.5 days (1–4). Mean duration of surgery was 7.2 h (5.7–8.6), with average blood loss of 134 ml/kg (36–256). Post-transplant, the INR and AST fell progressively in 8 children, with mean values at day seven of 1.16 (0.9–1.4) and 48.6 IU/l (33–105) respectively. One patient died from primary graft dysfunction and cerebral ischemia at 5 days post transplant. The other 8 children were discharged from hospital after an average period of 27 days (10–58) and are alive at a mean follow up of 22 months (range 15–64) with 6 patients having a follow up greater than 12 months. The function and regeneration of the native liver was assessed with Diisopropyl iminodiacetic acid scan (DISIDA), ultrasound and CT scan examination of native liver and allograft at 3 and 6 months after transplant. Ultrasound guided needle biopsies were performed at 6 monthly interval on native liver and allograft. The immunosuppression was tapered when there was evidence of native liver regeneration on DISIDA scan and liver biopsy. The native liver has fully regenerated in 2 children and was evidence of native liver regeneration on ultrasound at a mean follow up of greater than 2 years. Further studies showed a complete regeneration of the native liver and continued to be on standard autoimmune hepatitis immunosuppression. Conclusions: Auxiliary partial orthotopic liver transplantation in children with acute liver failure is a successful therapeutic option which allows complete regeneration of the native liver and withdrawal of immunosuppression in a high proportion of patients.

31 Familial adult haemochromatosis (HFE) gene: a familial HFE sequence?

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In the UK, over 95% of patients with genetic HC have mutations in the HFE gene, predominantly homozygosity for C282Y. Familial HC is due to a HFE mutation on one allele, although juvenile HC, with piutary and cardiac involvement, is reported. We describe two unusual pedigrees with familial hepatic iron overload, but no HFE mutation or features of juvenile HC. Family A: Two sons (age 35 and 37 yr) of a father with HC were screened and both found to have a high serum ferritin (4000 and 3800µg/l). Liver histology showed hepatic iron overload (liver iron concentration 15 µmol/g dry weight) with increased Kupffer cell iron in one. Two siblings of the father also have iron overload. Family B: In three generations, 75% of individuals tested have a high serum ferritin. On one side of the pedigree a sister (ferritin 4800µg/l) has daughters with a raised ferritin, hepatic siderosis (Kupffer cell and hepatocyte), normal routine haematology, but increased iron and possible mild dyserythropoiesis on bone marrow examination. The coding and splice site sequence and gene organisation for HFE in affected individuals from both families is normal, as is the coding sequence for beta-2-microglobulin. HLA-A and -B serotype does not segregate with iron overload. In summary, the iron overload in these families is not related to HFE mutations and appears to have dominant inheritance. Elucidation of the molecular mechanism responsible will be important in further dissecting pathways in iron metabolism, and in evaluating patients with haemochromatosis unrelated to HFE mutations.

32 Admission blood lactate is predictive of outcome in paracetamol induced acute liver failure

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Background: Paracetamol induced hepatoxicity is the most common cause of acute liver failure (ALF) in the USA and UK. The early identification of those patients who will not survive without emergency liver transplantation (OLT) is important to maximise graft availability before clinical deterioration precludes transplantation. Blood lactate may reflect both hepatic dysfunction and the degree of systemic tissue oxygenation. We have evaluated admission blood lactate measurements for the identification of patients in whom a fatal outcome is likely, in comparison with the currently applied clinical (King’s) selection criteria. Patients and methods: 101 consecutive patients with paracetamol induced severe hepatotoxicity admitted to intensive care were studied. Arterial lactate was determined at 4 hours (range 1.6–1.7) after admission using an automated enzymatic analyser (YSI 2300 Stat). Statistical evaluation utilised discriminating analysis based on multiple logistic regression. Threshold values were assessed by ROC curves. Of the 101 patients studied, 11 underwent OLT and were excluded from subsequent analysis. The remaining 90 patients included 54 who survived with a survival rate of 60%, and 36 who died. Median lactate was 1.4 mmol/l (0.5–7.9) in survivors and 7.8 mmol/l (1.7–20.1) in non-survivors (p<0.0001). On multiple linear regression, pH, temperature, mean arterial pressure, INR and creatinine were all independently associated with lactate (p<0.005). Admission lactate was the most powerful predictive cliner of outcome on multiple logistic regression (odds ratio 11.1, 95% CI 2.4 to 51.4). A cut-off of 3.5 mmol/l identified non-survivors with a sensitivity of 86% and specificity of 93% (overall misclassification rate 10%), as compared with a sensitivity of 74% and specificity of 90% for the applied King’s criteria (misclassification rate 16%). An admission lactate of greater than 3.5 mmol/l identified 9 of 36 (25%) non-surviving patients more than 24 hours before they fulfilled standard clinical criteria. Conclusions: Admission lactate identified non-survivors of paracetamol induced acute liver failure earlier than the currently applied clinical criteria, and with increased sensitivity and specificity.

33 Strong association between PSC and HLA-encoded gene MICA008 allele: is this the primary susceptibility allele?

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Background: Genetic susceptibility to primary sclerosing cholangitis (PSC) has been mapped between HLA-B and D. Other polymorphic gene loci within this region include MICA, a MHC class I related molecule. MICA is located between TNFα and HLA B, and encodes 151 codons of a cell surface glycoprotein. Recent studies suggest MICA may regulate protective responses by epithelial & T cells in the gut, and its expression has been described in stressed gastrointestinal epithelium. MICA expression could therefore indicate cell infection or transformation, and polymorphisms of the MICA gene have been strongly associated with chronic inflammatory disease. Aim: To determine the relative contribution of MICA polymorphisms in determining susceptibility to PSC. Methods: 60 PSC patients and 122 controls were genotyped for the sixteen alleles of the MICA locus using a polymerase chain reaction (PCR) based typing system which was developed in our laboratory. In the PCR reaction, MICA and the B2 Tissue Antigens 1999:53:167). Results: 86.2% of PSC patients have the MICA008 allele, and 65.5% overall were homozygous for this allele. In comparison, only 20.5% of controls had this allele, and this difference was highly significant (p 0.000005). Conclusions: PSC is strongly associated with homozygosity for the MICA008 allele. This ranks as one of the strongest genetic associations identified in PSC indicating that MICA008 homozygosity may be a high risk factor for PSC development. However, it remains to be confirmed if this is the primary genetic association with PSC.

34 Determinants of survival of chronic liver disease in the intensive therapy unit

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Background: Decompensated chronic liver disease (CLD) may be associated with high morbidity and mortality. Intensive therapy unit (ITU) admission in patients with CLD is not related to PSC. Methods: 60 PSC patients and 122 controls were genotyped for the sixteen alleles of the MICA locus using a polymerase chain reaction (PCR) based typing system which was developed in our laboratory. In the PCR reaction, MICA and the B2 Tissue Antigens 1999:53:167). Results: 86.2% of PSC patients have the MICA008 allele, and 65.5% overall were homozygous for this allele. In comparison, only 20.5% of controls had this allele, and this difference was highly significant (p 0.000005). Conclusions: PSC is strongly associated with homozygosity for the MICA008 allele. This ranks as one of the strongest genetic associations identified in PSC indicating that MICA008 homozygosity may be a high risk factor for PSC development. However, it remains to be confirmed if this is the primary genetic association with PSC.

Physiological parameters and survival: Markers indicative of respiratory dysfunction, liver, renal dysfunction, disordered acid-base balance, and sepsis, were all highly significantly associated.
with mortality in ITU. Similarly, physiological scoring for the first 24 hours of admission to ITU demonstrated significantly better scores for survivors compared to non-survivors (APACHE II score 18.5 ± 30, SAPS score 41 ± 66, APACHE III score 60.5 ± 108, p < 0.0001). The use of organ support and survival: Survival in the 24 patients who did not require organ support was 100%. 24 patients of the 29 (83%) who received ventilation alone survived. 3 of the 4 patients (75%) who received renal support alone survived. 3 of the 8 patients (38%) who received ventilation and inotropic support survived. 2 of the 8 patients (25%) who received ventilation and renal support survived. Mortality in the 27 patients who received ventilation, inotropic support and renal support was 100% (p < 10^-6).

Conclusions: Outcome of CLD in ITU is strongly related to the degree of organ dysfunction on admission, in particular hepatic and renal dysfunction, and is unrelated to the aetiology of CLD. Single organ support, particularly ventilation in the context of variecal haemorrhage, is associated with a good clinical outcome, all of whom had advanced disease. Fewer patients who require renal support survived: unless there is an acute reversible component to renal failure or liver transplantation is contemplated, the use of renal support in this patient group may not be appropriate. In selected cases, support of 2 organs may be justified, but higher levels of support would appear futile.

35 Suppression of hepatitis B and hepatitis C viraemia by concurrent delta virus infection: its beneficial effect on the outcome of orthotopic liver transplantation

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Introduction: Co-existing multiple hepatitis virus infections occur in a significant proportion of patients. The sequelae of these multiple viral infections is inadequately described with their influence on the outcome of orthotopic liver transplantation (OLT) not elucidated. Methods: Forty eight patients with dual or triple viral infections, in whom a liver transplant would be a possible outcome, were identified. These comprised patients with either simultaneous hepatitis B virus (HBV) and hepatitis C virus (HCV) infections (group 1, n=22), or HBV and hepatitis delta virus (HDV) infections (group 2, n=26). Of these, 8 patients in each group underwent OLT and were analysed separately for their clinical, histological and virological outcomes. Results: In the dual infection cohort (group 1), HBV DNA was present in 7/20 (35%) and HCV RNA in 10/16 (62.5%), whereas in the triple infection cohort (group 2) only 2/21 (9.5%) had detectable HBV DNA and no patient (0%) had HCV RNA viraemia (p values < 0.05). Of 39 patients with a complete serological profile, one had concomitant HBV and HCV viraemia. No difference in histological progression was evident in patients with group 1 with similar proportions requiring transplantation. Following transplantation, HCV recurred in four (50%) patients in group 1 and only one (12.5%) in group 2. In comparison, in a control group of transplanted patients with isolated HCV infection, there was universal recurrence. There was no case of HCV related graft loss or mortality in either of the groups. Patients with recurrence either had isolated HBV or HCV, but not simultaneous recurrence of the two.

Conclusions: In triple virus infections, HDV infection is associated with a suppression of both HBV and HCV viraemia. This suppression of HCV replication persisted despite immunosuppression following OLT.

36 Successful outcome of orthotopic liver transplantation in patients with pre-existing malignant states

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Introduction: Pre-existing malignancy is considered to be a relative contraindication to OLT because of the potential risk of recurrent or de novo tumors. The purpose of this study was to assess the outcome of OLT in patients with a pre-existent malignant state. Methods: Of 1097 OLTs performed between 1989 and 1998 at our centre, 12 (1%) had a pre-transplant malignancy. Of these 4 patients (33%) who required organ support alone survived. 3 of the 4 patients (75%) who received renal support alone survived. 3 of the 8 patients (38%) who received ventilation and inotropic support survived. 2 of the 8 patients (25%) who received ventilation and renal support survived. Mortality in the 27 patients who received ventilation, inotropic support and renal support was 100% (p < 10^-6).

Patients and methods: Eleven FAP patients were studied. TTR gene sequencing identified the Ala60 variant in 5 cases, Pro52 in 4 and Thr84 and Tyr77 in 2 cases each. Ten patients received OLT alone and the Tyr77 patient had a combined heart and liver transplant. Features of nephropathy and echocardiography findings were followed up in 16 patients pre- and post-O LT Results: Post transplant follow up echo appearances corroborated by histology in 4 cases, were suggestive of accelerated cardiac amyloidosis-post OLT in 8 of the 11 patients, all of whom had a pre-existing cardiac amyloid beforehand. Mean intraventricular septum (IVS) thickness increased from 14.22mm to 21.77mm within 1 to 4 months following OLT (p<0.05). There was no evidence of progressive cardiac amyloidosis in the single patient with normal echo pre-OLT, or the cardiac graft recipient with FAP/Tyr77. Seven patients died at 10 days to 36 months, the cause of death being HCC in 3/7, and either renal failure, sepsis, or malnourishment in 4. Motor function and general well-being improved in 3 patients and were unchanged or deteriorated post-OLT in the remaining cases. Overall survival was 75% reported by the FAP World Transplant Registry, and 90% in our own series. Around 70 other rarer TTR mutations also result in FAP but the outcome following OLT may be less favourable. Progressive cardiac amyloidosis after liver transplant is an unexpected and probably mutation dependent phenomenon that occurs in FAP patients with non-Met30 TTR variants. Aim: To describe the patients’ characteristics, cardiac findings and outcome of all FAP patients with known TTR mutations who underwent OLT at our centre. Patients and methods: Eleven FAP patients were studied. TTR gene sequencing identified the Ala60 variant in 5 cases, Pro52 in 4 and Thr84 and Tyr77 in 2 cases each. Ten patients received OLT alone and the Tyr77 patient had a combined heart and liver transplant. Features of nephropathy and echocardiography findings were followed up in 16 patients pre- and post-O LT.

Conclusions: Successful outcome of orthotopic liver transplantation in patients with pre-existing malignant states

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Animal models have demonstrated that Sirolimus has anti-proliferative activity in vitro and potent immunosuppressive activity in solid organ grafts; the latter finding has recently been confirmed in human renal transplantation. This combination of properties suggests it may have potential in patients undergoing liver transplantation for chronic liver disease in the presence of a pre-existent hepatic malignancy. The long term cardiac prognosis is of serious concern, however, the high mortality in this series was mainly compounded by severe nosocomial infections, renal failure, dysautonomia and malnutrition. TTR Ala60 is prevalent in Ireland and the role of very early OLT or combined heart and liver transplant will need to be defined.

38 A pilot study of Sirolimus (rapamycin) as primary immunosuppression following liver transplantation

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Sirolimus is an oral immunosuppressive agent that has anti-proliferative activity in vitro and potent immunosuppressive activity in solid organ grafts; the latter finding has recently been confirmed in human renal transplantation. This combination of properties suggests it may have potential in patients undergoing liver transplantation for chronic liver disease in the presence of a pre-existent hepatic malignancy.
Liver Transplant Unit and... Side-effects of Cyclosporin be...

Liver Institute, Menoufiya University, Egypt; Institute of Liver... glutamine, the major target antigen in porphyria cutanea.

Protein databases for homologies between unknown proteins and found 3 pairs of HCV and TPO motifs: PQRAXAXD\[T,S\]E, present in (a) HCV\textsubscript{1}, (b) TPO\textsubscript{1}, and (c) TPO\textsubscript{2}, respectively. Twenty- and 12-mer biotylated peptides spanning the motif-containing sequences and an irrelevant control peptide were constructed for future investigations. In HCV infected patients, double reactivity to TPO\textsubscript{1}, TPO\textsubscript{2}, and TPO\textsubscript{2}\textsubscript{2} was present in 2/14 (14%) cases without thyroid dysfunction and in 4/14 (29%) of those with thyroid dysfunction while in IFN\textsubscript{u}-treated patients, double reactivity was seen in 3/18 (17%) cases without thyroid dysfunction and in 11/18 (61%) of those with thyroid dysfunction. Double reactivity was present in 1/20 (5%) of patients with AIH and correlate their expression with a histological and biochemical activity.

Methods: We measured the number of cytokine producing cells, and the presence of antibodies to HCV and TPO peptides was tested by ELISA. Patients: A total of 64 HCV patients were studied: 36 on IFN\textsubscript{u}-treatment (median age 52, range 22–79, 24 females), 18 with and 18 without thyroid dysfunction; 28 not on IFN\textsubscript{u} (median age 54, range 25–92, 14 females), 14 with and 14 without thyroid dysfunction. Twenty pathologically tested and 20 normal demographically matched controls were also tested. Results: In HCV infected IFN\textsubscript{u}-untreated patients, double reactivity to at least one of the HCV and TPO peptide pairs was present in 2/14 (14%) cases without thyroid dysfunction and in 4/14 (29%) of those with thyroid dysfunction while in IFN\textsubscript{u}-treated patients, double reactivity was seen in 3/18 (17%) cases without thyroid dysfunction and in 11/18 (61%) of those with thyroid dysfunction. Double reactivity was present in 1/20 (5%) of patients with AIH and correlate their expression with a histological and biochemical activity.

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have previously reported that patients with sporadic poikilodermata cutanea tarda (PCT) have autoantibodies directed to recombinant human uroporphyrinogen decarboxylase (URO-D), an enzyme whose deficiency is central to PCT manifestations. To investigate whether anti-URO-D antibodies may be involved in impairing URO-D activity, we decided to define the linear epitope map of URO-D. A panel of twenty-five 20-mer biotinylated peptides, overlapping by 5 amino acids, spanning the entire enzyme molecule was constructed using F-moc solid phase chemistry (Chiron Mimotopes, Australia). Sera from 38 patients with sporadic PCT (34 males, 4 females, range 53 years, range 18-70 years), 26 of whom were positive and 12 negative for anti-URO-D antibodies, were tested in a sensitive ELISA, in which URO-D biotinylated peptides were added to the wells of microtiter plates coated with 5
diluted in 1/1000. Four epitopes were identified: URO-D16-35, URO-D196-215, which were recognised at a lower frequency by patient sera diluted in (3) 1/4 (3%), 1/2 (8%), and 1 (4%) autoantibody positive sera and by none of the autoantibody negative sera respectively. URO-D166-185, which were recognised in 9/15 (60%) of pathological but was absent in all the normal controls. Conclusions: These results show that: (1) linear autoantibody reactivity between Hepatitis C virus and TSH-R, the major thyroid antigen of Graves’ disease; (2) this is probably augmented by interferon-alpha treatment.

43 Cross-reactive immunity between hepatitis C virus (HCV) and thyroid stimulating hormone receptor, the major target antigen in Graves’ disease

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Beta-interferon (IFN-α) treatment. 45 High circulating levels of interleukin-6 and tumour necrosis factor-α are associated with hepatitis B virus clearance during alpha interferon treatment.

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It has been proposed that the balance between Th1/Th2 cytokines is central to the outcome of chronic hepatitis B virus infection and that Th1 predominance may contribute to recovery from disease and successful clearance of infection. The role of macrophage produced interleukin-6 and tumour necrosis factor-α and of TGF-β1 (Th3 cytokine) remains to be defined, even though we have previously reported that circulating levels of IL-6 were elevated in a small group of patients who seroconverted to anti-HBc during alpha-interferon (α-IFN) treatment (J Hepatol 1996;25:83). To investigate differences in the cytokine pattern of responders and non-responders to α-IFN therapy, we have measured circulating levels of IL-6 and TNF-α, IL-10 (Th2) and TGF-β1 using in house established ELISAs in 210 sequential serum samples from 100 untreated and 100 treated patients up to 7 years (median: 2.8 years) after α-IFN treatment from 25 children (median age 4.8 years, range 1.3-11.6 years, 20 boys) with chronic HBV infection. All patients had HBeAg positive before treatment. IFN-α dose was 5 MU/m² three times per week for 24 weeks. Thirteen became HBV DNA negative and anti-HBe positive, 12 did not. Normal ranges in pg/ml were established in 25 healthy children (median age 8 years, range 2-14 years, male:female: 10:15): IL-6 = 6 pg/ml (range 0–170), IL-10 = 10 pg/ml (0–20), TNF-α = 5 pg/ml (0–9) and TGF-β1 = 1 pg/ml (0–20). Baseline median concentrations were similar in responders and non-responders (1 pg/ml (range 0–35)), while during α-IFN treatment they increased from 3 to 35 times (median 10 times) around 200 times (median 777–3500) in responders. In contrast, little or no change in the IL-6 levels was observed in non-responders (389 pg/ml (0–1689), p<0.00005). Baseline levels of TNF-α were similar in responders and non-responders (163 pg/ml (0–80 pg/ml), while during treatment they were significantly higher in responders than non-responders (380 pg/ml (0–1702) v 242 pg/ml (0–1453), p=0.004). Baseline levels of IL-10 were similar in the two groups (924 pg/ml v
1126 pg/ml), but during treatment median IL-10 levels were higher in non-responders than in responders [1358 pg/ml (74-2580) vs 864 pg/ml (12-2844), p<0.001]. TGF-β1 levels did not differ significantly between the two groups at baseline or during treatment. There was a correlation between cytokine levels and serum viral load. Our results suggest that increased production of the macrophage derived TNF-α and IL-6 is associated with HBV clearance during o-IFN treatment, while increased production of the immunosuppressive Th2 cytokine IL-10 is associated with virus persistence. Cytokine therapy designed to enhance macrophage-derived cytokines and to inhibit IL-10 production may be beneficial in the treatment of chronic HBV infection. This work was supported by the Children’s Liver Disease Foundation, UK.

46 Stimulation of protein and DNA synthesis by ATP in hepatic stellate cell-conditioned medium of phospholipase D-meditated lipid hydrolysis

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ATP and related nucleotides released from injured cells and activated platelets may bind to cell surface receptors and function as paracrine-like ligands. A previous study has suggested that hepatic stellate cells (HSC) have nucleotide receptors that are coupled to inositol lipid hydrolysis, increased [Ca2+] and cell contraction (Takekura S et al. FEBS Lett 1994;354:53-6). We have shown previously in HSC that lipid hydrolysis is linked to the activation of phospholipase D (PLD) via PKC, and, (ii) the subsequent generation of phosphatidic acid (PA) from phosphatidylycholine plays a role in the mitogenic effect of PGDF. The aims of this study were therefore, first, to determine whether ATP and other nucleotides activate PLD in HSC, and, second, whether this activation is associated with mitogenesis assessed by [3H]DNA synthesis. All experiments were performed on transformed 2 week old HSC isolated from male Wistar rats after a 24h incubation in serum-free medium. Cells were preincubated with either [3H]choline or [3H]oleic acid to assess PLD activity, [3H]phenylalanine to determine protein synthesis or [3H]thymidine to measure DNA synthesis. ATP, ADP, UTP, UDP, CMP, CTP and GTP all elicited time- and concentration-dependent increases in [3H]choline release and [3H]phosphatidylethanolamine production, demonstrating activation of PLD. The magnitude of the response to different nucleotides was typical of the so-called “nucleotide receptor”. For ATP the response was first detected at 10µM and continued for approximately 15min. ATP stimulated protein synthesis in a concentration dependent manner when present for either 6 or 24h (20-25% increase at 100µM). DNA synthesis was also increased during the last 4h of a 24h incubation with ATP at concentrations up to 1000µM and this concentration dependence of the mitogenic effect on [3H]thymidine incorporation when added together with PDGF, the most potent HSC mitogen, ATP, and other nucleotides released by platelets and damaged cells during liver injury, may play a significant role in both the proliferation and contraction of HSC. The data presented suggest that these effects are mediated via the putative nucleotide receptor with the subsequent hydrolysis of inositol lipids and activation of PLD.

47 CTLA-4 gene polymorphism associated with autoimmune disease is a risk factor for advanced alcoholic liver disease

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The reasons why only a minority of heavy drinkers develop advanced alcoholic liver disease (ALD) remain unclear. But twin concordance studies have suggested that genetic factors may play a role. Clinical and laboratory studies have provided evidence that both cellular and humoral immune mechanisms may be involved in progression to pathogenesis, thereby implicating immunoregulatory genes as potential “candidates” determining genetic susceptibility to ALD. Cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) is a T cell surface molecule which competes with the co-stimulatory molecule CD28 for binding to the B7 ligands on APCs and is considered to influence the induction, maintenance and termination of the T cell response to antigen. CTLA-4 (~<) mice develop lethal autoantibody lymphoproliferative disease and an A→G polymorphism (Thr→Ala) in exon 1 of the CTLA-4 gene (IDDM12) is associated with susceptibility to autoimmune diseases such as diabetes and primary biliary cirrhosis (Agarwal et al. Hepatology 1998;28:40/4A). In this study we have looked for an association between this immunoregulatory polymorphism and ALD. We examined the frequency of the CTLA-4 A→G polymorphism in 287 patients with biopsy-proven advanced ALD (cirrhosis/ fibrosis), 92 heavy drinkers with normal liver function tests and 124 healthy controls. 67% of cirrhosis/fibrosis patients possessed at least 1 copy of the G allele versus 47% of controls (OR=2.1 [1.3-3.3], p=0.0041). The G allele frequency was 0.40 in cirrhose/firstic patients, 0.28 in controls (p<0.0003) and 50% of the heavy drinkers (OR:2.1 [1.3-3.3], p=0.0041). The G allele frequency was 0.40 in cirrhosis/fibrosis patients, 0.28 in controls (p<0.0003) and 50% of the heavy drinkers (OR:2.1 [1.3-3.3], p=0.0041). In conclusion, the G allele of the CTLA-4 exon 1 polymorphism previously associated with various liver and non-liver autoimmune diseases is strongly associated with advanced ALD. These findings provide direct evidence supporting a role for cellular immune mechanisms in the pathogenesis of ALD.

48 Hepatitis B virus (HBV) specific CD8 cells with diverse T cell receptor display limited flexibility in the recognition of a single HBV core epitope

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Hepatitis B virus infection, HBV, although a DNA virus, replicates via a reverse transcriptase activity of an RNA intermediate. It is therefore possible that other mutations arise within the genome leading to diversity of the viral population. Recently we reported a large outbreak of HBV infection in which 32 patients were found to be HBsAg positive; phylogenetic analysis linked this to a common source. We aimed to study the molecular evolution of the HBV genome during the incubation and clinical phases of acute and chronic hepatitis B, and to correlate viral diversity with the immunopathogenesis of this disease. From this outbreak, two patient groups were studied. Group 1 consisted of five patients identified early in the incubation phase leading to acute hepatitis B and subsequent clearance of HBsAg. Group 2 consisted of four patients who developed chronic hepatitis B, one of whom was the index case. We prospectively obtained serial serum samples and defined intervals for mutational analysis using denaturing gradient gel electrophoresis (DGGE). PCR was used first to amplify the core gene, following which the PCR products were cloned. Up to 96 colonies derived from each serum sample were obtained for DGGE to screen for viral heterogeneity and subsequent sequencing. We found during the incubation phase of infection, in which high levels of serum HBV DNA (10° copies/ml) were observed, that the early cloned samples of all Group 1 patients showed complete homogeneity, i.e. no variants of the core gene were seen after DGGE screening. Subsequent variation within the core gene, of up to 20% of the clonal sample population, emerged following the appearance of anti-HBc antibodies and the rise in serum ALT. Despite this variation, the dominant variant within the clonal sample population was detectable until HBV DNA became undetectable. Interestingly, following seroconversion to anti-HBs, which coincided with a decline in serum HBV DNA levels, variants diminished in proportion to the total HBV clonal population suggesting that the immune response was sufficient to control the emergence of viral sub-populations. In Group 2 there was no rise in ALT and no seroconversion to anti-HBe. In these
patients, despite high serum viral load when relapse error might be expected, viral diversity was only minimal. Thus, it is likely that variation within the core gene results from immune selection pressure especially during the acute phase of hepatitis B.

50 Hepatocyte lysis by HBV specific CD8+ cells may not be the principle mechanism of viral control in acute hepatitis B

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Background: Acute icteric hepatitis frequently follows hepatitis B virus (HBV) infection in adults. As HBV is non-cytotoxic in vitro, exposure to an infected hepatocyte by HBV specific CD8+ cytotoxic T cells is thought to be the central mechanism of liver damage and viral control after infection. The relationship between viral clearance, host immune response, and clinical disease was investigated in patients identified during the pre-clinical phase of acute hepatitis B. They had been exposed to HBV through a form of acupuncture, and the last possible date of infection was known for all patients.

Method: Five patients with pre-clinical acute HBV infection were studied. Sequential serum samples were analysed for HBV DNA by semiquantitative PCR, HBV serology, and ALC. HBV specific HLA-A2+ blood mononuclear cells were isolated, and the frequency of HBV specific CD8+ cells was monitored using HLA-A2 tetrameric complex. CD8+ cells were directly labeled to the T cell receptor of CD8+ cells specific for 3 distinct epitopes within HBV antigens. Analysis was performed by flow cytometry.

Results: Patients became jaundiced at least 10–14 weeks after exposure and all controlled infection. Peak levels of HBV DNA (mean 2.2 x 10^6 copies/ml) were seen during the pre-clinical phase, at which stage a low frequency of HBV specific CD8+ cells was demonstrated. Levels of HBV DNA then fell precipitously, asso- ciated with a virtual disappearance of HBV specific CD8+ cells from the circulation. Serum ALT levels peaked (mean 2409 U/L) after exposure, and all controlled infection.

Conclusion: A long term virological response was achieved in 84.6% of patients with a mean long term follow up of 10.9 months. These patients were treated for a mean of 8.4 months. The remaining 4 patients (15.4%) relapsed virologically (although transaminases remained normal) with a mean long term follow up of 11 months. There was no significant difference in genotype or duration of treatment in those patients who achieved a LTVR and those who failed to maintain a LTVR.

51 A long term response to alpha-interferon and ribavirin in hepatitis C is maintained in patients with a sustained response to treatment

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Background: Long term placebo controlled trials have recently shown that 40% of patients with chronic hepatitis C virus (HCV) infection treated with alpha interferon (α-IFN) in combina- tion with ribavirin achieve a sustained response (SR)—defined as undetectable HCV RNA by RT-PCR at least 6 months after the end of treatment. It is not yet known if these patients maintain a long term virological response (LTVR) beyond this time frame. For the pur- pose of this study long term response is defined as an absence of HCV RNA detectable by RT-PCR or branched chain DNA technology. Genotyping was performed in 63 patients using INNO-LiPA or R.F.L.P. Patients who achieved a SR were then further analysed to assess the LTVR to treatment.

Results: Results were unavailable for 12 patients who had moved out of the area or failed to attend for follow up. Of the remaining 51 patients, 27 had no response to combination therapy (LTVR) and 24 achieved an LTVR.

Conclusion: A LTVR after 22 months follow up. An end of treatment response was observed in 5 patients who failed to achieve a SR and in 2 patients who have been followed up for less than 6 months after treatment. A virological SR was achieved in 26/61 (42.6%) patients, in keeping with recently published randomised controlled trials. A LTVR was observed in 22 (84.6%) of these patients with a mean long term follow up of 10.9 months. These patients were treated for a mean of 26 months. The remaining 4 patients (15.4%) relapsed virologically (although transaminases remained normal) with a mean long term follow up of 11 months. There was no significant difference in genotype or duration of treatment in those patients who achieved a LTVR and those who failed to maintain a LTVR.

52 Inhibitory effect of SDZ-RAD on hepatitis B virus (HBV) protein production and transcription

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To study the potential effects of the hepatitis B virus (HBV) on host gene expression, in vitro, we used our novel HBV containing cell line in which the genome is maintained in a plasmid and a recently developed and highly effective method termed suppressive subtractive hybridisation (SSH). cDNA from the HBV transfected cell line was hybridised with an excess of cDNA from the plasmid transfected control cell line (without HBV) or “driver”. PCR amplification of target mole- cules generated a subtracted cDNA library representing differentially expressed genes. In order to identify genes which had been down regulated as well as up regulated, by HBV, the subtraction was also carried out in reverse. Subsequent cloning and analysis, by sequenc- ing, resulted in the identification of 192 genes of which 50 had 100% homology to a known sequence. 22 of these genes were upregulated and 28 genes were downregulated by the presence of the HBV genome in the cell line. To confirm the results obtained by SSH, two of the upregulated candidates were selected for further study: Glyceroldehyde-3-phosphate dehydrogenase (GAPDH) and human plasma membrane glycoprotein PC-1 (PC-1).
in the HBV transfected cell line, PC-1, which is important in cell cycle and may confer insulin resistance, was 18% higher. These results confirm the value of this approach. Confirmation and further study of the remaining 48 candidate genes identified by suppressive subtractive hybridisation could provide a deeper insight into the cellular interactions of the hepatitis B virus.

54 Tumour necrosis factor promoter allele-2 (TNF-2) and cytomegalovirus infection as liver-related risk factors for chronic rejection of liver grafts

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Chronic rejection of liver grafts is associated with elevated levels of the pro-inflammatory cytokine tumour necrosis factor-α. The TNF-2 promoter allele elicits elevated expression of TNF-α compared to TNF-1 and is in linkage disequilibrium with the extended haplotype HLA-A1-B8-DR3-DQ2 which has been associated with immune dysfunction and autoimmunity. Cytomegalovirus infection has also been associated with chronic rejection. We studied the effect of the promoter allele TNF-2, recipient HLA-DR3 status and CMV infection in relation to chronic rejection following liver transplantation. In an initial analysis, 123 liver transplant recipients (including 15 who later developed chronic rejection) were tested for TNF-α polymorphism allele status by PCR. Donor TNF-α allele status and recipient HLA-DR type were defined for 96 and 89 cases respectively. Confirmation was sought in an extended cohort of 307 recipients. All patients were monitored for CMV infection. Recipient HLA-DR3 (relative risk 3.41) and TNF-2 allele (relative risk 2.07) were risk factors for chronic rejection and increased the risk of chronic rejection synergistically with CMV infection (relative risk 5.01, OR 3.04 for HLA-DR3/ CMV infection and TNF-2 allele/ CMV infection respectively). Neither donor HLA-DR3 nor TNF-2 allele status were associated with chronic rejection. We conclude that the HLA-DR3 haplotype (which is associated with the TNF-2 promoter allele) and CMV are synergistic risk factors for chronic rejection of liver grafts.

55 High prevalence of osteoporosis in patients with chronic liver disease prior to transplantation

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Osteoporosis is a common and serious complication of liver transplantation, with post-operative fractures developing in up to 35% of patients. Although its pathogenesis is multifactorial, pre-existing bone disease is likely to play an important role. The aim of this prospective study was to evaluate bone mineral density in a large group of adult patients with chronic liver disease prior to transplantation. 243 patients (128 male & 115 female) with a mean age 53 years were studied over a 4 year period. Bone mineral density (BMD) measurements were made using dual energy X-ray absorptiometry at the spine (L1-4) and femoral neck (FN). Osteoporosis (T score <-2.5) at either L1-4 or FN was present in 37%, osteopenia (T score between -1 & -2.5) in 48% and normal BMD in only 15% of patients. The prevalence of osteoporosis was not significantly different in males and females (34.7% vs 39% respectively: P=0.442). Patients with cholestatic liver disease (PBC or PSC; n = 69) had lower BMD than those with non-cholestatic disease (e.g. alcoholic liver disease; hepatitis B or C; n = 173). Thus in the former group the mean + SEM T scores were -2.06 ± 0.16 and -2.20 ± 0.16 at the spine and femoral neck, compared with -1.40 ± 0.12 and -1.66 ± 0.13 respectively in the non-cholestatic group (p = 0.003). The lowest BMD values were found in the five patients with cysctic fibrosis, with mean T scores at the spine and hip of -4.07 ± 0.69 and -3.20 ± 0.56. A logistic regression model was used to look for independent risk factors for osteoporosis. Increasing age and lower body weight were found to be significant independent risk factors, whilst diagnosis, sex and menopausal status (in females) were not. These results demonstrate a high prevalence of osteoporosis in patients with chronic liver disease prior to transplantation. Our findings emphasise the importance of prophylactic measures in such patients to optimise bone health before transplantation and reduce the risk of subsequent fractures.

56 Vitronectin induces migration of activated T lymphocytes: a mechanism for lymphocyte infiltration of hepatic tumours

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Background: The mechanisms by which lymphocytes infiltrate hepatic tumours are poorly understood. The extracellular matrix glycoprotein vitronectin is a particularly significant component of the stroma of hepatic tumours and might thus play a role in the recruitment and retention of infiltrating leukocytes. In order to address this hypothesis, we examined the ability of vitronectin to support migration of peripheral blood T lymphocytes (TIL) isolated from hepatic colorectal cancer metastases. In addition we investigated the possibility that this interaction was mediated by the integrin αvβ3. Methods: TIL and PBL migration to vitronectin (2.0·00002µg/ml) was assessed using an in vitro chemotaxis assay and T cells expanded in culture for up to 14 days. The same cells were analysed by fluorescence microscopy to determine whether ligation of the vitronectin receptor resulted in cytoskeletal rearrangement and actin polymerisation. Vitronectin was presented in soluble and immobilised states. Expression of αvβ3 integrin on leukocytes was determined by flow cytometric analysis. Results: TIL from hepatic tumours and activated PBL migrated to vitronectin in both its soluble and immobilised states. Maximal levels of migration to vitronectin correlated with upregulation of the integrin αvβ3. In contrast, although TIL also migrated to vitronectin this was not mediated by the integrin αvβ3. Fluorescence microscopy demonstrated a conversion from cortical actin in unstimulated cells to discrete localisation of actin filaments when stimulated with vitronectin implying that engagement of the vitronectin receptor results in modifications to the cytoskeleton enabling the cells to migrate on the substrate. Summary: 1) Vitronectin can trigger both chemotaxis and haptotaxis of activated T lymphocytes. 2) T cells infiltrating liver tumours show minimal αvβ3-dependent migration whereas activated blood T cells respond in an αvβ3-dependent manner. This raises the possibility that TIL can act as an alternative vitronectin receptor.

Conclusions: Vitronectin is a potent pro-migratory factor for activated T cells. It may be particularly important for regulating lymphocyte migration through the extracellular matrix in hepatic tumours.

57 Evidence for opposing effects of the Jun family of proto-oncogenes on TIMP-1 promoter activity in activated rat hepatic stellate cells

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Activation of the hepatic stellate cell (HSC) has been shown to be a key event in liver fibrosis. This event involves the transdifferentiation of the quiescent or non-activated HSC into a proliferating myofibroblast-like cell that is responsible for deposition of most of the excess extracellular matrix found in the fibrotic liver. One of the most profound biochemical changes associated with HSC activation is the induction of the tissue inhibitor of metalloproteinase 1 (TIMP-1) gene. As there is accumulating evidence for an important role for TIMP-1 in liver fibrosis (Iredale et al, J Clin Invest 1998;102:538–49), we are interested in determining how transcription of the TIMP-1 gene is regulated in HSCs. Previous work showed that an AP-1 binding site was present in the TIMP-1 promoter. In the current study we confirm this idea by showing that co-infection of a TIMP-1 promoter CAT reporter construct with anti-sense JunD oligonucleotides resulted in reduced Timp-1 expression whereas activated blood T cells showed that an AP-1 binding site was essential for TIMP-1 transcriptional activity on September 17, 2023 by guest. Protected by copyright.