Liver F218-F227

ANTINEUTROPHIL CYTOPLASM AUTOANTIBODIES (ANCA) AGAINST BACTERICIDAL/PERMEABILITY-INCREASING PROTEIN IN PRIMARY SCLEROSING CHOLANGITIS.

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Background: In primary sclerosing cholangitis (PSC) the ANCA target antigen remains obscure. Bactericidal/permeability-increasing protein (BPI) has been described as an ANCA antigen in cases of systemic vasculitis where the target antigen is neither myeloperoxidase (MPO) nor proteinase-3 (PR3). BPI has high affinity for gram negative bacterial lipopolysaccharide and attenuates its pro-inflammatory action on monocytes and endothelial cells. Aim: To investigate the prevalence and clinical relevance of anti-BPI ANCA antibodies in PSC. Methods: Sera were studied from 34 patients with PSC (20 prior to liver transplantation), 25 patients with primary biliary cirrhosis (PBC), 11 with autoimmune hepatitis (AIH) and 30 patients with non-autoimmune biliary tract disease. Indirect immunofluorescence for ANCA was performed on alcohol fixed neutrophils, and solid phase ELISA for PR3, MPO, Cathepsin-G, lactoferrin and BPI antibodies. Results were analysed with respect to extent, stage of disease and history of cholangitis.

Results: ANCA against the BPI antigen were negative in all sera. Antibodies against other ANCA antigens were rare. In PSC there was no significant association between ANCA or anti-BPI status and cirrhosis, progression to transplantation, presence of IBD, extent of involvement of the biliary tree or history of cholangitis. In the control group there was a significant association between a history of biliary sepsis and both ANCA positivity and the presence of anti-BPI antibodies (p=0.05). Conclusion: Antibodies to BPI account for a minority of the ANCA in the autoimmune liver diseases. In controls both the presence of anti-BPI and ANCA antibodies are related to a history of sepsis in the biliary tract.

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SERUM HYALURONIC ACID LEVELS RELIABLY PREDICT THE PRESENCE OF CIRRHOSIS IN PATIENTS WITH CHRONIC HEPATITIS C INFECTION.

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Elevated levels of Hyaluronic Acid (HYA) are associated with sinusoidal capillarisation, for example in hepatic cirrhosis. Liver biopsy is important in assessing severity and progression of disease in chronic hepatitis C virus (HCV) infection particularly with respect to alpha-interferon therapy. The aim of this study was to evaluate the role of serum HYA in detecting cirrhosis in patients with HCV infection compared with cirrhosis of other aetiologies.

Methods: Serum HYA was measured, using a radiometric assay (Pharmacia, Sweden) in 69 patients with HCV infection and 152 patients with chronic liver disease of other aetiologies (alcohol, n=70; autoimmune chronic active hepatitis, n=23; primary biliary cirrhosis, n=17; cryptogenic, n=15 and various, n=27).

Results: HYA levels (um/l) were significantly higher in patients with cirrhosis of the liver (n=127; mean 440, 95% CI 367-515) (p<0.0001) compared with hepatic fibrosis (n=23; mean 144, 95% CI 69-160), chronic hepatitis (n=60; mean 63, 95% CI 37-91) and fatty liver (n=11; mean 107, 95% CI 37-177). Within the cirrhotic population, there was no significant difference in HYA levels between different aetiologies: primary biliary cirrhosis (mean 500, 95% CI 323-678), alcoholic cirrhosis (n=61, mean 498, 95% CI 366-631), autoimmune chronic active hepatitis (n=12; mean 225, 95% CI 122-328), cryptogenic cirrhosis (n=14; mean 225, 95% CI 122-328), and chronic hepatitis C infection (n=15; mean 386, 95% CI 260-570).

Conclusions: Measurement of HYA levels can reliably differentiate cirrhotic from non-cirrhotic liver disease of different aetiologies. This is of important clinical significance in chronic HCV infection; for example in HCV infected haemophiliacs or in HCC surveillance.
CYP2E1-DEPENDENT ACTIVITIES IN NEEDLE LIVER BIOPSY SAMPLES - CORRELATION WITH BIOCHEMICAL AND PATHOLOGICAL INDICES

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The development of alcoholic liver cirrhosis occurs only in susceptible individuals and is not always correlated with alcohol intake. CYP2E1 is the principal alcohol metabolising enzyme in the heavy drinker and polymorphism of the CYP2E1 gene (rs1799939) and part restriction sites may be implicated in the development of cirrhosis. CYP2E1-dependent activity was quantified by chlorozoxazone 6-hydroxylation in small samples (3-14mg tissue wet weight) obtained during routine diagnostic liver biopsies from 18 patients. 6-hydroxy chlorozoxazone formation could be detected after incubation with microsomal protein at levels as low as 10 μg. The samples showed a 10-fold range in activity (0.11-1.17nmol/min/mg protein). CYP2E1-dependent activity was positively correlated with serum gamma glutamyltransferase (r=0.82, p<0.0001) and with alcohol intake (r=0.66, p=0.0029). There was no correlation of chlorozoxazone 6-hydroxylation with smoking habits of the patients. Alcohol intake was desigated 1-4, 1 corresponding to an intake of <0.5 units per week, 2 was 3-14 units, 3 was 14-21 units per week and 4 was 21+ units per week. The mean value for chlorozoxazone 6-hydroxylation was 0.33 ±0.16 mmol/min/mg protein in group 1, 0.24 ± 0.05 in group 2, 0.37 ±0.17 in group 3 and 0.83 ±0.35 in group 4. Individuals with the highest CYP2E1 activity and alcohol intake had histological features suggestive of alcoholic cirrhosis. Quantifying CYP2E1-dependent activity in small samples obtained by needle biopsy provides a technique which will allow direct correlation of hepatic enzyme activity with genetic polymorphisms of CYP2E1.

EXPRESSION OF ENDOThelial ADHesION MOLECULES AND T-CELL hOMING RECEPTORS IN HEPATIC TUMOURS

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Lymphocyte-mediated mechanisms play an important role in local anti-tumour immunity, although the degree to which different tumours are infiltrated by T-cells varies. Before lymphocytes can enter tumour they must first recognise and bind to adhesion molecules on tumour endothelium and the local expression of these molecules will control the composition of the infiltrate. In the present study we demonstrated that the mononuclear cell infiltrate in hepatic tumours was composed predominantly of T-cells. We then looked for the expression of T-cell homing molecules on tumour infiltrating lymphocytes (TIL) and potential counter-receptors on tumour endothelium, that might determine the intensity and subset composition of the infiltrate in primary hepatocellular carcinomas (HCC) and colorectal hepatic metastases (CRM).

Methods: Immunohistochemistry and flow cytometry were used to study L-selectin and the integrins LFA-1, α4β7, α4β8 and VLA-4 on T-cells; tumour endothelium was stained and scored on a scale of 0 (absent) to 3 (strongly expressed) for ICAM-1, VCAM-1, E-selectin and vascular adhesion protein-1 (VAP-1), a novel endothelial adhesion molecule that we have recently shown supports T-cell binding to normal hepatic endothelium.

Results: In HCC (n=5) T-cells comprised 12.5 +/−3.4% of total nucleated cells in the parenchyma and 23.4 +/−1.6% in the stroma. Overall 49% of the inflammatory infiltrate were T-cells, 67% of which were CD4 T-cells. In contrast, the infiltrate in CM (n=5) comprised 6.9 +/−0.8% of parenchymal cells and 12.1 +/−2.2% of stromal cells with 39% of the inflammatory infiltrate composed of T-cells evenly divided between CD4 and CD8 subsets. HCC-derived TIL expressed high levels of LFA-1 and VLA-4 whereas CM TIL showed low VLA-4 expression. The T-cell homing molecules L-selectin, α4β7 and α4β8 were expressed by TIL in either tumour. There were marked differences between endothelial adhesion molecules for the two tumours. In all 5 cases endothelium showed strong expression of ICAM-1 (mean score 2.3 ±0.22) and vascular adhesion protein-1 (mean score 0.5 ±/−0.5) compared with weak ICAM-1 expression (mean 0.9 ±/−0.38 ±0.01) and almost absent VAP-1 expression in CM. Endothelium in both tumours expressed VCAM-1 weakly (HCC 0.25 ±/−0.0.2; CM 0.3 ±/−0.25) but not E-selectin.

Conclusions: 1) HCC are more heavily infiltrated with TIL than CM.
2) The low levels of α4β7, α4β7 & L-selectin on HCC and CM-infiltrating T-cells suggest these molecules are not involved in TIL homing to liver tumours.
3) The high level of ICAM-1 and VAP-1 on endothelium in HCC may explain why these tumours are more heavily infiltrated by T-cells than CM.
4) That HCC and CM differ in their expression of endothelial adhesion molecules suggests that local tumour factors, rather than the anatomical site of the tumour, determine the phenotype of tumour endothelium.

LIVER TRANSPLANTATION FOR AUTOIMMUNE HEPATITIS - A SINGLE CENTRE EXPERIENCE

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Introduction. Auto-immune hepatitis (AIH) is a common indication for liver transplantation (LT). We review our Unit's experience over the past 12 years.

Patients. Between Dec 1983 and Sept 1995, 53 patients (13M:40F, median age 39 years) were transplanted for AIH. Median length of follow up is 46 months.

Results. The actuarial 10 year survival was 64% with 39 patients currently alive. Eight patients needed a second graft (5 for chronic rejection, 2 for hepatic artery thrombosis, 1 for biliary stricture) and 2 patients needed three grafts (for recurrent chronic rejection in both cases). The 12 early deaths post-LT were predominantly due to infection, including fungal septicaemia. The duration of immunosuppression prior to LT did not influence survival. Thirty three patients have been followed up for >6 months. Unexplained chronic hepatitis (CH) in the graft was seen in 20/33 (18 mild CH, 2 moderate CH) with associated transaminisits in 11 cases. CH was diagnosed within 24 months of LT in 19/20 cases. Follow-up autoantibody data was available in 18/33 patients - autoantibody persistence occurred in 13, non-persistence in 5. Autoantibody persistence was not related to graft rejection or CH. HLA B8 and DR3 was found frequently (~50%) in the liver recipients. Neither recipient nor donor HLA B8/DR3 status influenced incidence of acute rejection episodes or unexplained CH post-LT. However, chronic rejection was less common in patients receiving a HLA B4+ve/D3+ve graft.

Conclusion. Survival following LT for AIH is similar to that for other conditions. CH occurring within 2 years of LT is a frequent occurrence and is of uncertain significance. CH with associated transaminisits may represent recurrent disease in some patients.
CARDIAC SPECIFIC ALPHA-MYOSIN ANTIBODIES IN ALCOHOLIC LIVER DISEASE: AN IMMUNOLOGICAL ROLE IN ASYMPTOMATIC LEFT VENTRICULAR ENLARGEMENT?

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Asymptomatic left ventricular enlargement (LVE) occurs in up to 30% of patients with alcoholic liver disease (ALD). The presence of IgG antibodies to cardiac acetylcholine adducts in alcohols suggest that immunological mechanisms may play a role in the pathogenesis of LVE. We postulate that other antigens such as cardiac myosin may also be important in the development of LVE.

Aims Our first aim was to determine if cardiac specific anti-alpha myosin antibodies are present in the sera of ALD patients. Secondly we investigated if these antibodies are associated with echocardiographic and signal-averaged electrocardiographic (SAECG) abnormalities.

Methods Anti IgG alpha myosin antibodies were assayed in 51 consecutive ALD patients attending a liver clinic (age 52 ± 9.9, 36 males) and 92 patients with ischaemic heart disease (age 56 ± 7.6, 70 males), and in 203 normals (age 45 ± 16, 100 males) using an ELISA.

Results Anti IgG myosin antibodies were present in 9/51 (18%) patients with ALD (P < 0.0001, x²), 4/92 (4%) patients with ischaemic heart disease (P=0.44) compared to 4/203 (2%) normals. Myosin antibodies were significantly more common in ALD (P=0.02) than ischaemic heart disease patients. LVE was detected in 25% of patients with ALD and the SAECG was abnormal in 17%. Anti myosin antibody positivity did not correlate with LVE or SAECG abnormalities in ALD patients.

Conclusion Myosin antibodies are significantly increased in patients with ALD. They are not common in those patients with established ECHO or SAECG abnormalities suggesting they do not occur simply as a consequence of myocardial damage. Myosin antibodies may be an early predictor of future development of LVE in patients with ALD.

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CELL LINES DERIVED FROM NORMAL HUMAN LIVER BY INFECTION WITH A RETROVIRUS CONTAINING THE SV40 LARGE-T ANTIGEN

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Background Many of the available human liver cell lines are tumour-derived and this limits the effectiveness of such cells as models of normal tissue. Introduction into normal cells of a temperature-sensitive (ts) variant of the SV40 large-T-antigen produces cell lines which proliferate at 33.5°C but cease proliferating and enhance differentiated function at 39.5°C.

Method Primary cultures of normal, mixed, primary human liver cells were infected with an amphotropic mouse retrovirus containing the SV40 large-T-antigen and a selectable marker (neo). We report here the early results of morphological and functional characterization of the cells derived from these cultures.

Results Five non-clonal cell strains have been generated so far. Four have been in culture for three months and a fifth for ten months. Morphologically, all strains have a mesenchymal pattern of growth. All cells possess nuclear T-antigen. The doubling times at 33.5°C range between 37 and 480 hours. Culture at 39.5°C retarded cell growth (e.g. from 190 hours at 33.5°C to 450 hours at 39.5°C) but did not completely arrest growth. Albumin secretion was not demonstrable using ELISA. Immunostaining demonstrated that approximately 1% of cells in each strain were positive for cytokeratin 18 (a parenchymal cell marker) and 1% for von Willebrand factor (an endothelial cell marker); up to 20% possessed α-smooth muscle actin (a hepatic stellate cell marker). Moreover, HGF mRNA has been demonstrated in the one strain (the oldest) tested so far by Northern blotting.

Conclusion Five cell strains have been generated. From the morphology, staining pattern, ELISA and Northern blot results, the cultures are composed principally of immortalized hepatic stellate cells.

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DO PHARMACOLOGICAL AGENTS FOR PORTAL HYPERTENSION COMPROMISE RENAL FLOW?

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Both propranolol (PROP) and isoborcide-5-mononitrate (ISMO) are commonly used in the management of portal hypertension. Recently, concern has been expressed that these drugs may compromise renal function in cirrhotic patients by reducing renal perfusion as a result of their hypotensive action. We assessed the haemodynamic effects including renal blood flow of these drugs.

Methods: 12 cirrhotic patients were studied. After an overnight fast, heart rate, mean arterial pressure (MAP), hepatic venous pressure gradient (HPVG), axiogenous flow (AZY) and unilateral renal vein flow (RBF) (by direct reverse thermodilution method) were recorded. Patients were studied with PROP (5 patients), mean Child-Pugh score (CPS) 9.2 (1.3) or 20mg ISMO (6 patients, mean CPS 9.7 (1.7)) and the above measurements repeated after 60 minutes.

Results: There was no correlation between the baseline RBF and CPS although it was significantly reduced in patients with severe ascites (Pt0.01). Haemodynamic parameters at baseline (pre) and at 60 minutes (post) are summarised below:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pre</th>
<th>Post</th>
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<tr>
<td>PROP</td>
<td></td>
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</tr>
<tr>
<td>MAP</td>
<td>80.5(4.9)</td>
<td>78.7(4.3)</td>
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<td>ISMO</td>
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Results are expressed as mean (standard error) with pressures in mmHg and flow in ml/min. (*p<0.05, **p<0.01, ***p<0.001)

Conclusion: Despite the anticipated reduction in other haemodynamic parameters, RBF did not fall significantly following administration of either PROP or ISMO to cirrhotic patients.

IBD and colorectal F228-F238

INCREASED EXPRESSION OF CIRCULATING LEUCOCYTE ADHESION MOLECULES CD11b AND CD18 IN INFLAMMATORY BOWEL DISEASE. CE Collins, C Davies*, MG MacHey*, DA McCarthy*, DS Rampton. Gl Science Research Unit and Department of Haematology*, St Bartholomew's and The Royal London School of Medicine and Dentistry, Whitechapel, London.

Adhesion of leucocytes to vascular endothelium depends in part on their expression of specific surface antigens. Amongst these, CD11 and CD18 bind to endothelial cell intercellular adhesion molecules-1 and -2. Leucocyte activation is a feature of inflammatory bowel disease (IBD) and monocyte-endothelial cell interaction may be an early event in pathogenesis. Conventional therapy including sulphalazine may reduce expression of leucocyte adhesion molecules. We tested the hypothesis that IBD is associated with up-regulation of circulating leucocyte adhesion molecules.

METHODS: Using fluorescently-labelled specific monoclonal antibodies and a novel flow-cytometric method, we measured leucocyte surface expression of the antigens CD11a, b and c and CD18 in unixed whole blood in Crohn's disease (CD), ulcerative colitis (UC) and healthy controls.

RESULTS: No statistically significant differences in % cells positive for these antigens were detected, but for CD11b and CD18, mean fluorescence intensity (MDI), a quantitative measure of antigen density, was increased for granulocytes (GNLs) and monocytes (MNs) in IBD.

MFI expressed as median (interquartile range):

<table>
<thead>
<tr>
<th>Antigen</th>
<th>GNLs</th>
<th>MNs</th>
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<tbody>
<tr>
<td>CD11b</td>
<td></td>
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<tr>
<td>CD18</td>
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</tbody>
</table>

Healthy controls 8

healthy controls 8

active CD 10

inactive CD 11

active UC 11

inactive UC 11

CD18

9 (5-11) 1 (1.1)

69 (5-100) 49 (5-100)

62 (15-98) 50 (15-98)

69 (15-98) 50 (15-98)

62 (15-98) 50 (15-98)

69 (15-98) 50 (15-98)

*P<0.05 vs controls, **P<0.005 vs controls

CONCLUSIONS: Increased expression of the adhesion molecules, CD11b and CD18, on circulating granulocytes and monocytes is likely, by enhancing leucocyte adhesion to vascular endothelium, to promote cellular diapedesis and infiltration of the bowel wall in IBD.