Mutations in a putative hepatitis B HLA A1 CTL epitope affect HLA binding and correlate with disease severity

C J HEALEY, J CHRISTIE, M E LAI, V CERINDOLO, R W CHAPMAN, W M C ROSENBERG* (Nuffield Department of Clinical Medicine, Oxford and Ospedale Microcorticin, Cagliari, Italy) A vigorous peripheral blood cytotoxic T-cell (CTL) response is associated with viral clearance in acute HBV infection but intrahepatic CTL may mediate hepatocyte damage in chronically infected subjects. Escape from immune detection is thought to be present in infected patients. In a family chronically infected with HBV with differing outcomes we have studied the effect of mutation within a core HLA-A1 restricted CTL epitope on HLA-A1 binding and disease course.

HLA core protein antigen was sequenced in viral isolates from HLA-typed family members. Synthetic peptides based on the HBV sequences were tested for binding to HLA-A1 molecules in vitro using an HLA class I assembly assay. Binding was correlated with disease course.

One patient with a mutation in the A1 epitope, which abolges HLA-A1 binding and three who lack HLA-A1 had no liver disease. Two patients with mutations that increased binding and one patient without mutations developed chronic liver disease.

A benign course of HBV infection may result from an unrecognized mutation in HLA A1 binding residues that abrogate binding.

CTL epitope mapping in HCV using HLA assembly and CTL assays

C J HEALEY, S MACADAM, M PLEBANSKI, R W CHAPMAN, W M C ROSENBERG* (Nuffield Department of Clinical Medicine, Oxford) Cytotoxic T lymphocytes (CTL) clear acute viral infections but may facilitate viral persistence through selection of variants that escape immune detection. Immune escape may arise by loss of peptide binding to HLA molecules or through failure of CTL recognition. We have established assays of HLA binding and CTL recognition to map HCV immune epitopes.

(a) The HCV genome was scanned for HLA-B7 binding motifs (Xxxxxxxx). Nine HLA-B7 peptides and seven control HLA-A2 peptides were each incubated with radiolabeled HLA molecules. Binding affinity was determined by isoelectroimmun precipitation with the conformational specific Mab (W6/23). (b) CTL were cultured from peripheral blood (with peptide, IL-2, IL-7, and KLH) and tested for their ability to lyse autologous peptide-pulsed target cells.

All control HLA-A2 peptides bound. Seven of nine candidate B7 peptides bound to HLA-B7, of which one HLA-B7 peptide, identifying a new epitope, and an HLA-A2 control epitope were recognised by patient derived CTL.

We have used an HLA assembly to identify seven new HLA-B7 HCV epitopes and have shown CTL recognition of at least one.

An analysis of factors affecting post-transplant lymphoproliferative disorder (PTLD) following orthotopic liver transplantation (OLT)

M MCCARTHY, J RAMAGE, JE GANE, B PORTMANN, M RELA, H HEATON, A FAGLIUCI, G MUPPI, ROGER WILLIAMS* (Institute of Liver Studies, Liver Transplant Surgical Service, and Department of Haematology, King’s College Hospital, London, UK) Over a 15-year period at this unit, 10 adult liver allograft recipients (six females, four males) developed PTLD three to 36 months post-OLT (median 22.2 months), an overall prevalence of 2.1%. Mean age was 47.1 years (range 24 to 56). Sites of primary lesions were: liver graft three of 10, kidneys two of 10, ovaries one of 10, brain one of 10, lungs two of 10, pancreas one of 10, bone marrow two of 10, and lymph nodes four of 10. All tumours were B-cell lymphomas: six lymphoblastic, three immunoblastic, and one Hodgkin’s lymphoma. Clonality was assessed by both immunohistochimistry and gene rearrangement studies: four were monoclonal, three polyclonal, and three undetermined. Epstein-Barr virus (EBV) serology (EBNA, VCR antigens), EBV PCR, and in situ hybridisation (ISH) were performed: six of 10 were positive for EBNA antigens and seven of 10 had EBV detected in liver tissue by PCR or ISH. Monoclonal tumours were treated with systemic chemotherapy, polyclonal with combinations of immunosuppression withdrawal and acyclovir. Mortality is 60% and those alive have a mean survival of 720 days (range 600 to 1440).

PTLD is presenting earlier post-OLT and continues to have a high mortality. Exposure to EBV plays a significant part in tumour development and efforts must be made directed towards reducing the risk of exposure to the virus early post-OLT.

The haemochromatosis gene lies more than 3 Mb telomeric to HLA-A

J D SHEARMAN, J J POINTON, C STONE, A MERRYWEATHER-CLARKE, W M C ROSENBERG*, K JH ROBBON for the UK Haemochromatosis Consortium (Nuffield Department of Clinical Medicine and MRC Haematology Unit, Oxford) The molecular defect in haemochromatosis (HC) remains undefined. Genetic linkage analysis has placed the gene close to HLA-A on chromosome six and is a candidate for positional cloning. The candidate region for the gene covers several megabases, extending from HLA-F to D6S399. The disease is strongly associated with the microsatellite marker D6S105, which maps telomeric to HLA-A within this region. We are analysing linkage of HC to newly generated microsatellite markers close to D6S105 to narrow the candidate region in an attempt to identify the gene.

We have constructed a yeast artificial chromosome (YAC) contig extending 1.5 Mb telomeric to HLA-A beyond the telomeric limit of the MHC and towards D6S105. A second contig centred on D6S105 spanning 3 Mb does not overlap the proximal contig. New microsatellites isolated from all show strong allelic association with the disease and locate a peak of association 700 kb telomeric to D6S105. Although haemochromatosis shows strong linkage to HLA-A these studies show that the gene is more than 3 Mb telomeric to this locus. These findings will permit identification of the disease locus.

Hepatic expression of gelatinase A is increased during progressive liver fibrosis

R C BENTON, S FAWLEY, C J HOVELL, J F IRDEALE, M J P ARTHUR (University of Medicine, Southampton General Hospital, Southampton) Gelatinase A released from hepatic stellate cells (HSC) activated after liver injury may degrade normal basement membrane and facilitate fibrogenesis by disturbing HSC and hepatocyte function. We have examined this hypothesis by quantifying hepatic expression of gelatinase A in two rat models of progressive fibrosis. Hepatic fibrosis was induced by bile duct ligation (BDL) or CCI, administration twice weekly. Some rats were given CCI before four weeks then left to recover for four weeks. RNAase protection assay quantified gelatinase mRNA content of total liver RNA. Gelatinase mRNA was increased at 4 weeks (383%) and three days (400%) after BDL. In the CCI model, gelatinase mRNA was 157% of control at 24 hours, 455% (72 hours), 295% (one week), and 25% (four weeks). However, this expression fell during CCI recovery. At day 0 recovery, gelatinase mRNA was 537% of untreated controls, falling to 201%, 210%, 111%, and 148% at three, seven, 14, and 28 days recovery. HSC isolated from normal rat liver showed increasing expression of gelatinase mRNA during activation in vitro by culture on plastic. By northern blotting, gelatinase/β-actin mRNA ratio was 0.999 in freshly isolated HSC and 0.322, 0.758, 0.903 at five, seven, and 10 days of culture respectively. We conclude that progressive liver fibrosis is associated with increased hepatic gelatinase mRNA expression and that activated HSC may be an important source of this enzyme.

Analysis of the TIMP-1 promoter activity in freshly isolated and activated hepatic stellate cells

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Cambridge) Activation of hepatic stellate cells (HSC) is central to the process of hepatic fibrosis. When activated, HSC produces large amounts of tissue inhibitor of metalloproteinases-1 (TIMP-1), which downregulates the matrix-degrading activity of many matrix metalloproteinases. The aim of this study was to examine the regulation of the promoter of the human TIMP-1 gene in HSC. We therefore adapted several methods of transient DNA transfection into eukaryotic cells for the use in HSC. Electroporation was a very reproducible method of transfection of activated HSC. However, because of the high numbers of cell death no transfection of freshly isolated HSC could be obtained. Calcium phosphate mediated transfection worked well for both freshly isolated and activated HSC. Different promoters including viral (SV40, HSV thymidine kinase), eukaryotic (β-actin), and artificial sequences (AP-1, NFκB) linked to CAT reporter genes were found to be active in HSC. The 5’ region of the human TIMP-1 gene was isolated, sequenced, and cloned into the pBLCAT3 vector. TIMP-1 promoter activity was found upregulated in activated HSC. By the use of promoter truncations, further sequence elements have been identified. In conclusion, TIMP-1 activity in HSC is regulated at the promoter level.

Identification of a potential novel regulatory protein in Kupffer cell activation

D HOU, S I FRIEDMAN, A ALAZAR, M J P ARTHUR, P J WINTHORNE (University Medicine, Southampton General Hospital, Southampton, *Liver Center Laboratory, University of California, San Francisco) In liver injury Kupffer cells (KC) undergo phenotypic changes in response to a programme of gene induction termed activation. Although central to the pathogenesis of liver injury, little is known about transcriptional regulation of KC activation.

cDNAs that are induced in rat KC (freshly isolated) 2 to 4 hours after administration of CCl4, were identified by a subtractive hybridisation/differential screening approach. cDNAs deemed activation specific were cloned into suitable vectors for DNA sequencing and expression analyses. Full length cDNAs were isolated using marathon RACE.

One cDNA identified, KC2, has a 270 bp motif (78% homology) common to human ETO (MTGB) protein, a putative zinc finger transcription factor in myeloid cell types. Marathon RACE yielded a 3 kb DNA in the 3’ direction with a poly A tail. Sequence data obtained to date show no homologies to known genes (BLAST). RNASe protection analyses showed induction of the transcript of KC2 in freshly isolated KC after CCl4, peaking at 12 hours. Northern analysis of whole liver RNA, using the 3 kb RACE product as a probe, detected a regular transcript maximal at 24 hours, not identified in normal liver RNA.

We have identified a novel cDNA in KC, which is induced early after CCl4, liver injury. Sequence analysis shows homology to a myeloid transcription factor, ETO.

Interaction between cytokine activated killer cells and human intrahepatic biliary epithelial cells: modulation by ICAM-1 blockade

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Human intrahepatic biliary epithelial cells (HIBEC) constitute a major immunological target during liver allograft rejection and primary biliary cirrhosis. This has been attributed to their capacity to express immune cell ligands during inflammation. Initial experiments showed that cultured HIBEC after stimulation with pro-inflammatory cytokines increased their expression of ICAM-1 and their capacity to bind mononuclear cells.

Standard 51Cr-release assays were used to assess the capacity of cytotoxic effector cells to damage cultured HIBEC. IL-2 activated natural killer (LAK) cells were used in this experiment to bypass the requirement for antigen specificity. Cultured HIBEC were stimulated for four days with pro-inflammatory cytokines (IFNγ or TNFα) for one hour; LAK cells were then added for four hours. Anti-ICAM-1 antibody was added to come cultures to modulate LAK cell adhesion to ICAM-1 expressing HIBEC.

Addition of LAK cells at an increasing effector:target (E:T) ratio caused an increasing release of 51Cr, a ratio of 200:1 produced a maximum specific 51Cr release of 38% (p<0.001). Anti-ICAM-1 produced a significant reduction in the amount of 51Cr released from stimulated HIBEC and also in the amount of 51Cr released from HIBEC.

These results show that the capacity of HIBEC to upregulate their expression of ICAM-1 during activation is associated to their susceptibility to lytic damage by cytotoxic effector cells. These findings suggest a potential option for therapeutic intervention.

Increased expression of monocyte chemotactic protein-1 in alcoholic liver disease

N C FISHER, S A AFFORD, P BRUN, C MORLAND, A KEOGH, J PEAR, S G HUBSCHER, H D ADAMS (Liver Unit, Queen Elizabeth Hospital, Edgbaston, Birmingham B15 2TH) Alcohol abuse in man is a major factor in the tissue damage of alcoholic liver disease (ALD) the mechanisms of leucocyte recruitment are unknown. We investigated the expression and regulation of monocyte chemotactic protein-1 (MCP-1), a potent monocyte and T-lymphocyte chemotactic factor, in ALD to discover if MCP-1 is involved in alcohol induced inflammation.

MCP-1 protein and mRNA expression were analysed in liver biopsy specimens using immunohistochemistry and in situ hybridisation. MCP-1 production by human hepatocytes was tested in vitro after exposing hepatocytes to TNFα, ethanol or acetaldehyde.

Tissue expression of MCP-1: In normal liver MCP-1 protein and mRNA were detected weakly on endothelial cells within portal tracts. In alcoholic hepatitis there was intense expression of MCP-1 protein and mRNA in balloononed hepatocytes at areas of inflammation. In alcoholic cirrhosis MCP-1 protein and mRNA were upregulated in leucocytes and endothelial cells. Bacterial Hepatocyte secretion of MCP-1: Untreated hepatocytes or hepatocytes exposed only to ethanol or acetaldehyde did not secrete MCP-1. TNFα treated hepatocytes secreted MCP-1 (mean 115 pg/ml); concomitant treatment with acetaldehyde augmented the effects of TNFα induced MCP-1 secretion (mean 215 pg/ml); whereas concomitant treatment with ethanol reduced it (mean 44 pg/ml).

(1) Expression in areas of inflammatory damage and fibrosis suggests a role for MCP-1 in the pathogenesis of ALD. (2) Anergic monocytes of acetaldehyde NP+ on hepatocytes suggests a mechanism for MCP-1 upregulation in ALD.

Alcoholic hepatitis is associated with upregulation of redox sensitive neutrophil chemotactic cytokines

J MALTFY, G BIRD, W RIGGITT, N SHERON (University of Southampton, School of Medicine, Southampton General Hospital) Interleukin 8 (IL-8), a neutrophil chemokine, is increased in alcoholic hepatitis. In this study values of the other major neutrophil chemotactic protein GROα were measured with two monococyte specific chemokines RANTES and MIP1α were assayed by ELISA in liver homogenates.

GROα was significantly increased in alcoholic hepatitis compared with normal and disease controls, and values of MIP1α associated significantly with those of IL-8 (r=0.47, p<0.001). In contrast, mean values of the monocyte chemokine RANTES were slightly lower in the AH group compared with other disease controls, and values of MIP1α were not significantly increased in any of the disease groups. These data provide further evidence of a role for redox sensitive neutrophil chemokine activation in the pathogenesis of the unique immunological injury associated with alcoholic intake.

IgA reactivity to intestinal bacterial isolates in alcoholic liver disease

A C DOWDS, T A POULTON, J D MAXWELL (Division of Medicine and Immunology, St George’s Hospital Medical School, London SW17 0RE) Alcoholic liver disease is accompanied by an increase in serum IgA due to increased production by B cells. The anti- gen stimulus that determines this response is unknown. We speculated that increased gut permeability in alcoholic liver disease allows intestinal bacterial antigens access to the portal circulation and stimulates IgA production. We investigated the IgA reactivity to a range of gut and non-gut bacterial isolates in alcoholic and other liver diseases using immunoblotting.

Eleven of 15 (73%, p<0.001) alcoholic cirrhotic patients had positive reactivity to Bacteroides, 11 of 13 (85%, p<0.001) to E coli, and 10 of 13 (77%, p=0.05) to Clostridium perfringens. No responses were seen in PBC, other liver cirrhotic patients, or alcoholic patients. One of 12 (8%, p=NS) alcoholic cirrhotic patients had positive reactions to Staphylococcus aureus compared with none of the other groups.

The results show that a high proportion of patients with alcoholic cirrhosis have IgA antibodies to Bacteroides, E coli, and Clostridium perfringens but not to Staphylococcus aureus. These antibodies seem to be specific to alcoholic liver disease. Intestinal bacterial antigens may account for the IgA response in alcoholic liver disease.
Neutrophil superoxide (O{'2}-) and hydrogen peroxide (H2O2) production in patients with acute liver failure (ALF)

M CLAPPERTON, N ROLANDO, L SANDOWAL, ROGER WILLIAMS (Institute of Liver Studies, King’s College School of Medicine and Dentistry, Bessemer Road, London SE5 9PJ) Neutrophil superoxide and hydrogen peroxide production, essential for bacterial killing, was assessed in ALF patients. Isolated patient neutrophils were stimulated by zymosan opsonised with either pooled control serum (control) or autologous patient serum (patient zymosan). Control neutrophils were stimulated with control zymosan.

Superoxide: 21 ALF patients (14 paracoxal overdose (POD); seven other aetiologies) and 17 controls were tested. Superoxide was significantly lower for ALF POD neutrophils stimulated either by control zymosan (median 432.00 nmol/10^6 cells (range 257-942), p<0.05) or patient zymosan (median 542.00 nmol/10^6 cells (range 287-699), p<0.05) compared with control neutrophils (median 687.00 nmol/10^6 cells (range 245-976)). The difference in superoxide production between neutrophils and control neutrophils was not significant. Hydrogen peroxide: 12 ALF POD patients and 14 controls were tested. Hydrogen peroxide from ALF neutrophils stimulated with either control zymosan (median 0.98 nmol/10^6 cells (range 0.02-12.65), p<0.005) was significantly less than control neutrophils (median 8.00 nmol/10^6 cells (range 2.40-19.00). The degree of encephalopathy, renal failure, and infection did not influence superoxide or hydrogen peroxide production. Overall, a cellular and opsonic defect leads to reduced superoxide and hydrogen peroxide production due to POD and could increase susceptibility to infection in ALF patients.

Plasma concentrations and hepatic mRNA expression of transforming growth factor-β1 in patients with fulminant hepatic failure

Y MDWA, P M HARRISON, P G LANGLEY, R D HUGHES, ROGER WILLIAMS (Institute of Liver Studies, King’s College School of Medicine and Dentistry, Bessemer Road, London SE5 9PJ) The impaired liver regeneration seen in severe aetiological fulminant hepatic failure (FHF) could be due to production of an inhibitory factor like transforming growth factor-β1 (TGF-β1). The aim of this study was to investigate plasma concentrations and hepatic mRNA expression of TGF-β1 in patients with FHF. Plasma concentrations of TGF-β1 measured by ELISA in FHF patients on admission (median 38.8 ng/ml, range 8.4-491 ng/ml, n=57) were significantly higher than those in control subjects (23.0 ng/ml, 8.5-34.9 ng/ml, n=20, p<0.001). Significantly higher plasma concentrations were seen in NABN hepatitis patients (57.9 ng/ml, range 7.4-180 ng/ml, p<0.005) compared with paracetamol overdose (POD) patients (37.1 ng/ml, 8.4-72.5 ng/ml, n=47, p<0.001). In liver tissue, northern blot analysis showed increased mRNA expression of TGF-β1 in POD (n=8, p<0.05), but not in NABN hepatitis (n=6), compared with controls (n=4), suggesting that the liver is not the only source of the increased plasma TGF-β1 in NABN hepatitis. The mRNA expression of H3 histone, a marker for liver proliferation, was significantly increased in POD (p<0.05) compared with the controls, but not in NABN hepatitis. The increased circulating plasma TGF-β1 could be a factor in the impaired liver regeneration and related to poor prognosis in FHF due to NABN hepatitis.

Auxiliary partial orthotopic liver transplantation (APOLT) for acute liver failure (ALF)

S P FERRERA, M MCCARTHY, A J LILLIS, J WENDON, M BELA*, N HEATON*, ROGER WILLIAMS (Institute of Liver Studies, and *Liver Transplant Surgical Service, King’s College Hospital, Denmark Hill, London SE5 9RS) APOLT holds potential advantages over conventional OLT, e.g., the experience of the technique in ALF is limited. We describe our initial experience in seven patients (four males, three females, mean age 26, range 14-35 years) with ALF (paracoxal three, NABN two, autoimmune one, eosinothy, one) who fulfilled KCH criteria. On admission, the median values for the INR, creatinine and bilirubin were 7 (3.4-15.3), 2.5 μM (1-5.5) and 320 (70-648) μM, respectively. The reasons for performing APOLT, rather than OLT, were macroscopic evidence of native liver regeneration (n=5) or significant psychiatric history (n=2).

All patients received ABO matched left (n=2) or right (n=5) auxiliary grafts. Median duration of surgery was 8.5 (7.3-10 hours), with blood loss of 8-31 (4.6-14.6 litres). Post-transplant, the INR and AST fell progressively in all patients, with median values at day 7 of 1.4 (1.0-2.4) and 108 (78-910 IU/l). Three patients died from sepsis on days 7, 10, and 30. At two weeks post-APOLT, HIDA scans and bronchial scans showed the remaining four patients showed partial regeneration of the native lobe in two. At three months, one patient had complete native lobe regeneration, and immunosuppression has since been withdrawn completely.

Although patient selection remains poorly defined, APOLT in ALF is technically feasible and, in some patients, permits native liver regeneration and eventual immunosuppression withdrawal.

F2-isoprostanes increase portal pressure

RICHARD MARLEY, DAVID HARRY, RADHI ANAND, KEVIN MOORE (Academic Department of Medicine, Royal Free Hospital) The F2-isoprostanes (a group of prostaglandin-like compounds, formed by non-enzymatic peroxidation of arachidonic acid) are generated during oxidative stress, and have previously been shown to cause pronounced vasoconstriction. Current evidence suggests that these could act through a thromboxane-like receptor, as their actions may be blocked by thromboxane receptor antagonists. We have previously shown that these compounds may be generated during hepatic devascularisation. We have tested the hypothesis that 8-iso-PGF2α (a major F2-isoprostanate formed in vivo) could increase portal pressure.

The liver from normal or bile duct ligated cirrhotic rats was perfused with Krebs-Henseleit buffer in a non-recirculating system at a constant flow rate. 8-iso-PGF2α was infused at 1.0, 2.5, and 10 nmol/min, and portal pressure monitored continuously. Infusion of 8-iso-PGF2α at 2.5 and 10 nmol/min into normal liver increased portal pressure from a baseline of 8.2 (0.5) mm Hg (mean (SEM)) to 9.3 (0.8) and 10.0 (0.8) mm Hg respectively (p<0.05, Wilcoxon test). At 1 nmol/min, there was no significant change in portal pressure. There was a noticeably increased sensitivity of BDL cirrhotic to 8-iso-PGF2α. Portal pressure increased from 11.4 (0.8) mm Hg to 12.0 (1.0) mm Hg and 17.7 (2.3) mm Hg when 8-iso-PGF2α was infused at 1.0, 2.5, and 10 nmol/min respectively (p<0.01). Preliminary studies have shown that the portal hypertensive effect of 8-iso-PGF2α can be blocked by SQ 29,548, a thromboxane receptor antagonist.

We conclude that 8-iso-PGF2α may cause pronounced changes in the portal pressure in cirrhotic rats. If extrapolated to humans, then medication of oxidative stress (for example, by reducing or alcoholic binges) may increase the formation of F2-isoprostanes (e.g., 8-iso-PGD2α) and thus increase portal pressure.

Factors related to early mortality after transjugular intrahepatic portosystemic shunts (TIPS) for uncontrolled variceal haemorrhage

Y NIKOLOULOU, D PATCH, P A MCCORMICK, G MATTHEWS, R DICK, A ARMONGS, G WANNEMETHES, A K BURROUGHS (Hepato-biliary and Liver Transplantation Unit, Royal Free Hospital Hampstead NHS Trust, London NW3) Uncontrolled variceal haemorrhage is the main indication for TIPS. However, mortality is 50% for this high risk group. We have evaluated clinical and laboratory variables before TIPS to establish predictors of mortality.

Over a three year period 54 patients failed sclerotherapy for acute variceal bleeding and had an emergency TIPS. Failure of sclerotherapy for resistant varices (for example, bleeding after two injection sessions (n=45), or bleeding from gastric/ectopic varices (n=9). Some 33 variables were analysed from data available immediately before TIPS.

Child/Pugh grade was A7, B19, C28. Four patients continued to bleed after TIPS. Twenty four patients died within six weeks. Average follow up was 335±32.4 days. In a multivariate analysis using backward step regression, five factors had a significant prognostic value: moderate/severe ascites (p<0.03), requirement for mechanical ventilation (p<0.003), log WBC (p<0.0002), log platelets (p<0.003), and log PT TK (p<0.004).

A prognostic index (PI) score was derived, where presence of moderate/severe ascites, or need for ventilation, scored 1: PI=1(34 ascites)+1(ventilation)+2(02 log (WBC)+132 (PLT)+1(95 log (PTTK)). Using this equation, 45% (n=10) of deaths occurred in the fifth quintile (PI=9-3), where mortality was 100%.

Patients with uncontrolled variceal haemorrhage have a high mortality despite immediate control of bleeding by TIPS, particularly when associated with markers of advanced liver disease, ascites, and multi-organ failure. The use of TIPS is therefore not justified in this subgroup. The reliability of this prognostic score should be validated prospectively.

Is staging in primary biliary cirrhosis accurate?

M C GARRIDO, S G HUBSCHER (Department of Pathology, University of Birmingham) Our aim was to evaluate sampling variability of liver
biopsy specimens in patients with primary biliary cirrhosis (PBC).

We examined sections from 50 PBC liver specimens obtained at transplantation. The degree of fibrosis was assessed on a scale of 0–4 using two methods (a) simulated needle biopsy assessing fields approximately the size of a conventional needle biopsy and (b) whole section scanning identifying areas with the least and most severe degrees of fibrosis.

With the method of whole section scanning only 10 cases (20%) had a consistent degree of fibrosis in all the sections scanned. By contrast the same stage was assigned in 30 cases (60%) examined by the simulated needle biopsy method. Comparison of the results obtained by the two methods showed a discrepancy of one or two stages in 33 cases. This resulted from discovering areas with a lesser degree of fibrosis in whole sections compared with the simulated needle biopsy samples.

There is considerable variation in the degree of fibrosis in livers with PBC even in end-stage specimens obtained at transplantation. Simulated needle specimens give more consistent results. This may be a reflection of the procedure applied when assessing liver biopsy specimens where the greatest degree of abnormality is used for determining the stage. Staging of PBC in small needle biopsy specimens is valuable as long as the appearances are interpreted with caution bearing in mind that there is considerable variability in stage.

'True' positive AMA and normal alkaline phosphatase: is this primary biliary cirrhosis (PBC) – 10 years on, the answer is yes

J V METCALF, O F W JAMES, J M PALMER, M F RASSENDINE, D E JONES, H C MITCHESON (Department of Medicine, University of Newcastle upon Tyne NE2 4HH) In 1985 we reported 29 patients with positive AMA and normal LFT's in whom histology was diagnostic/compatible of PBC in 24, normal in two. We now report 10 year follow up. Notes of all 29 were examined, five had died, all other patients were seen again. Median follow up since first AMA was 14.7 years (10.1–22.5). Five patients died, none of liver disease, median age 78 (72–83). Twenty four had developed persistent raised alkaline phosphatase, one jaundice (0.9–19 years, median 5.6) after +ve AMA. Twenty two (75.9%) developed typical PBC symptoms (pruritus, malaise, persistent upper abdominal pain). Repeat histology was available in 10 (after median 11.4 years). In nine histology was diagnostic/compatible in both biopsy specimens, four had progressed in histological stage. The tenth was normal in both biopsy specimens. Baseline sera were tested against PDC and OgDC (ELISA) in 27. Twenty one were +ve, 20 of these had diagnostic/compatible biopsy specimens. In the six ELISA negative patients only one had a compatible biopsy.

'True' (ELISA +ve) AMA +ve patients with normal LFT's almost certainly have early PBC, confirmed on histology. In this group although disease progression was very slow over 75% developed typical PBC (symptoms, cholestatic LFT's) within 10 years.