CLINICAL SIGNIFICANCE OF P53 EXPRESSION IN OESOPHAGEAL CANCER.

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The tumour suppressor gene p53 is believed to play an important role in the progression of human malignant tumours through mutation or over-expression. To examine the clinical significance of the expression and accumulation of P53 in oesophageal cancer, 10 formalin fixed paraffin-embedded, specimens of oesophageal cancer were analysed immuno-histochemically using a monoclonal antibody ( DO-7, DAKO)and microwave oven heating method. Cell proliferation index for all tumours was calculated from immunostaining with the MIB-1 monoclonal antibody and correlated with clinical parameters as well as p53 status.

Of the 100 tumours 39% were adenocarcinoma, 54% squamous cell carcinoma, 3% oat cell carcinoma and 4% undifferentiated. 29% of all the tumours were P53 negative 71% p53 positive with a mean positivity percentage of 59.4 SD 25.4. The age, sex, site, tumour differentiation, lymph node status, distal metastases, treatment and survival was correlated to p53 status and cell proliferation index. Correlation's were found between p53 status / proliferation index (p<0.001), treatment, stage and survival (p<0.005). Overall the cumulative survival rate of patients with p53 expression was lower than that of the patients without expression (P<0.05). The prognostic value of p53 appears to be highly significant in surgically resected cases of squamous cell carcinoma (p<0.02).

IMMUNOLOGY

OXIDATIVE STRESS IN ACUTE PANCREATITIS

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Acinar cell injury in acute pancreatitis leads to an increased production of oxygen free radicals (OFRs). These may cause local and distant tissue damage by increasing capillary permeability. OFRs are scavenged by intracellular antioxidants and serum factors, principally albumin, urate, ascorbate, α-tocopherol, and retinol. A balance normally exists between the serum total antioxidant capacity (TAC) and OFRs. Potential for damage arises when the production of OFRs rises beyond the TAC.

TAC was measured by colourimetry in 23 patients with acute pancreatitis, on admission and daily for 5 days. Serum albumin and urate were also measured, as components of TAC. Malondialdehyde (MDA), a lipid fragment production caused by OFR peroxidation of phospholipid membranes, was measured as an indicator of oxidative stress. A control group consisted of 22 patients undergoing elective surgery. Results are expressed as medians [interquartile range]; statistical analysis was by Mann-Whitney U Test.

TAC was significantly lower in the pancreatitis group than the preoperative values for the control group, both on admission (1.4 [1.3-1.6] vs 1.6 [1.4-1.8] mmol/l, p=0.016) and on the day of the lowest measurement (1.3 [1.2-1.4] mmol/l, p=0.001, day 3). Both albumin (34 [31-37] vs 37 [34-41] g/l, p=0.017, on day 1; 31 [25-35] vs 37 g/l, p=0.003, on day 3) and urate (243 [192-266] vs 381 [308-446] mmol/l, p=0.001, on day 1; 210 [192-281] vs 381 mmol/l, p<0.001, on day 3) were also lower in the pancreatitis group than controls. MDA was higher in the pancreatitis group than in the control group (1.41 [1.2-1.3] vs 1.208 [0.8-1.9] on day 1) but this did not reach statistical significance.

These results indicate that serum TAC is reduced in acute pancreatitis. TAC may be insufficient to counterbalance increased OFR production, resulting in organ damage caused by oxidative stress. If low levels of TAC are found to correlate with organ damage, then its measurement may permit the identification of a patient group who might subsequently benefit from antioxidant therapy.

CBD-ASSOCIATED T CELLS ARE OLIGOClonal, SHOW CLONAL PERSISTENCE AND CYTOLYTIC ACTIVITY

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The phenotype of T lymphocytes associated with the common bile duct (CBD) is unknown. We investigated the hypothesis that they behave like other intraepithelial lymphocytes (IEL). We determined the phenotype, cytotoxic potential and T cell receptor (TCR) repertoire of T cells obtained during endoscopic retrograde cholangiopancreatography (ERCP). Three subjects were studies: two with primary sclerosing cholangitis (one as a complication of ulcerative colitis) and a third normal control. After establishing a short-term T cell line, cells were 1) stained with monoclonal antibodies for flow cytometric analysis, 2) used as effector cells for cytotoxicity and redirected lysis assays and 3) analyzed for TCR-β chain transcript expression. Flow cytometry revealed, that for all the subjects, 98% of the T cells were TCR-β positive with a CD4: CD8 ratio that ranged from 0.5 to 1.2. Redirected lysis studies showed that the normal CBD-derived T cell line had cytotoxic potential, that did not differ from that of a normal peripheral blood T (PB) T cell line; however, when the intestinal epithelial cell line, Caco-2, was used as a target the CBD-derived T cell line exhibited significantly more cytolytic activity in comparison to the control PB T line. CDR3-length displays suggested that all three CBD-derived lines were oligoclonal. This was confirmed by sequencing of PCR amplification products after using TCR-β chain specific primer pairs; TCR-β chain sequences were reiterated in all Vβ-specific clones analyzed. In one case, two expanded TCR-β chains could be identified which were persistent in the bile duct over a period of one year. We conclude that the human common bile duct contains T cells which share several characteristics with intestinal IELs.

SEMI QUANTITATIVE POLYMERASE CHAIN REACTION (PCR) FOR CYTOMEGALOVIRUS (CMV) DNA IN SERUM IDENTIFIES ADENOMAS OF CMV RELATED DISEASE FOLLOWING LIVER TRANSPLANTATION. R.C Evans, A.Soin, T.Wrighthtit, GJM Alexander. University of Cambridge School of Medicine, Cambridge, UK, CB2 2QQ

CMV infection is common following organ transplantation including the liver with a clinical spectrum ranging from asymptomatic to life threatening illness. PCR for CMV DNA has been used to monitor active infection and is exquisitely sensitive. However a positive result is not necessarily indicative of symptomatic disease. To investigate whether a semi quantitative PCR could be adapted to identify patients with active infection at risk of clinical illness, we have performed PCR for CMV DNA in serial samples of urine or serum in 32 patients undergoing liver transplantation. PCR was conducted alongside exogenous plasmid controls using a fluorescent labelled sense primer; the products were quantified using an ABI 373A automatic DNA sequence (Genescan 1.1 software). Of 6/32 patients, both donor and recipient were sero negative for CMV and all samples (n=65) were PCR negative. 16/26 patients with a sero positive recipient and/or donor had CMV replication detected by PCR of urine (n=11) and serum (n=12) -5 of whom were not identified by conventional virological diagnosis. The clinical illness (pyrexia and/or hepatitis) was recognised in 7/7 patients PCR positive in urine with >2.5 x 10^5 genome equivalents/ml in contrast to 0/5 with less than 2.55 x 10^5 (p<0.02). However estimation of viral load by semi quantitative PCR in urine was clinically unhelpful. Semi quantitative PCR of serum for CMV DNA is more sensitive than conventional diagnosis and can be used to identify patients with CMV infection who would benefit from anti viral therapy.
GOBLET CELL HYPERPLASIA IN PARASITE-INFECTED MICE IS REGULATED BY A TH1 TO TH2 SWITCH.
N. Ishikawa, Y.R. Mahida, D. Wakelin. Dept. of Life Science and Division of Gastroenterology, University of Nottingham, Nottingham, UK.

Altered goblet cell activity is associated with intestinal conditions such as inflammatory bowel disease. We have used a parasite infection that generates immune-mediated changes in intestinal goblet cells to examine this regulation.

NIH inbred mice were studied 4 & 8 days after infection with the nematode Nippostrongylus brasiliensis. Intestinal tissue from control and infected mice was examined histologically and goblet cells quantified per 10 villi. Worms per intestine were also determined. ELISA was used to measure cytokine production from mesenteric lymph node lymphocytes after in vitro stimulation with ConA. Interferon-gamma (IFN-y) and interleukin-5 (IL-5) were used as candidate TH1 and TH2 cytokines. Five infected or control animals were studied at each time point and data are expressed as mean±SEM.

Infection induced an increase in goblet cell numbers, which rose from 103±14.2 in controls and 102.6±5.6 at day 4 to 174.8±18.6 at day 8 (P<0.05). The increase in goblet cell numbers corresponded to the time of worm loss, the number of worms at days 4 and 8 being 190.7±27.5 and 64.4±15.8 respectively. Levels of IFN-y rose from 20.3±3.6IU/ml in controls to 131.4±24.2IU/ml on day 4 (P<0.01), but then declined to 21.4±3.8IU/ml on day 8. IL-5 levels remained low in controls [24.7±3.4 IU/ml] and on day 4 [67.3±2.5 IU/ml] but rose sharply to 265.4±43.3 IU/ml on day 8 (P<0.01).

Invasion of the small intestine by N. brasiliensis induces marked increases in goblet cell numbers. These changes are known to be immune-dependent. The close correspondence of goblet cell hyperplasia with a switch from TH1 to a TH2 response in local lymphocytes suggests a direct role for TH2 cytokines in regulating goblet cell numbers. Data from other experiments, using the nematode Trichinella spiralis, show a similar picture, implying that TH2 cell regulation of goblet cells may represent a general response to intestinal insult.

CYTOKINES IMPAIR MICROSMAL ENZYME DETOXIFICATION IN ISOLATED HEPATOCYTES: IMPLICATIONS FOR A BIO-ARTIFICIAL LIVER.
P. McCloskey, R Boulton, D Calnan, C Selden and H Hodgson. Royal Postgraduate Medical School, London.

Background. The prognosis of acute liver failure remains bleak. Temporary support for patients by a hybrid biological / artificial liver (BAL) is an enticing prospect for therapy. Since pro-inflammatory cytokine networks are activated in liver failure, and augment parenchymal liver injury they could also impair cell function in a BAL.

Aims. To assess the detoxification potential of primary hepatocytes exposed to cytokines with and without insulin and dexamethasone - agents that have previously been shown to modulate these activities.

Methods. Primary rat hepatocytes isolated by collagenase perfusion were plated on collagen coated microtitre plates in William’s E medium 5% FCS, ± dexamethasone (10⁻⁷ M) ± insulin (10⁻⁶ M). Cells were incubated for 18 hours in the presence of IL-1B (10 ng/ml), IL-6 (10 ng/ml), TGFβ (0.1 ng/ml) or TNFα (10 ng/ml). Cytochrome P450 1A1 and 1A2 activities were assessed after 1 hour incubation with ethoxy- or methoxy-resorufin respectively. Free resorufin was measured by fluorescence at 530/590 nm (Ex/Em).

Results. Each cytokine significantly suppressed enzyme activity by between 17% and 86%. IL-1 showed the most profound depression of both basal (86%) and dexamethasone (83%) treated cells. In general 1A1 and 1A2 activities were altered in parallel. Dexamethasone and insulin enhanced cytochrome P450 1A1 and 1A2 activities but were unable to reverse cytokine inhibition, except by TNFα where 1A2 activity was partially restored from 27% to 87%.

Conclusion. The cytokines implicated in liver failure depress the endogenous cytochrome P450 detoxification systems in isolated hepatocytes. Failure to metabolize toxins intrinsic to the fulminating state could be detrimental to clinical outcome. Dexamethasone and insulin will not abrogate this effect. Strategies to remove cytokines will be necessary in the development of a BAL.

HEPATOCYTES FROM REGENERATING RAT LIVER ARE MORE SENSITIVE TO GROWTH INHIBITION BY IL-1: IMPLICATIONS FOR THE CONTROL OF LIVER REGENERATION.
Ralph Boulton, Denis Calnan, Clare Selden, Thames Hodgson. Royal Postgraduate Medical School London W12 0NN

Background. 70% partial hepatectomy initiates a rapid and co-ordinated wave of cell proliferation that involves most hepatocytes. The mechanisms terminating cell division are not clear, but paracrine interactions between liver cell subpopulations are important. TGFβ inhibits hepatocyte proliferation and has been proposed as a paracrine regulator of hepatocyte proliferation. Paradoxically, hepatocytes from regenerating liver become refractory to the inhibitory influence of TGFβ. We have previously demonstrated that IL-1α and IL-1β are secreted by non-parenchymal liver cells isolated from regenerating liver and these also suppress hepatocyte proliferation and that mRNA for these cytokines are modulated in regenerating liver.

Aims. To determine the sensitivity of regenerating hepatocytes to growth inhibition by IL-1.

Methods. Hepatocytes were prepared from young male rats by collagenase perfusion from unoperated liver or 24 hours after 70% partial hepatectomy. DNA synthesis was stimulated by insulin (10⁻⁶ M) with HGF, TGFβ or EGF or with or without IL-1 (0-10 ng/ml) and assessed by 3H-Thymidine incorporation.

Results. For all mitogens regenerating hepatocytes were considerably more sensitive to the growth inhibitory effects of IL-1 than resting cells. For example results expressed as % of maximal incorporation for TGFβ were:

<table>
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<tr>
<th>Hepatocytes</th>
<th>Resting hepatocytes</th>
<th>Regenerating hepatocytes</th>
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<tr>
<td>IL-1α 10 ng/ml</td>
<td>78.8 ± 10.0</td>
<td>31.6 ± 6.0</td>
</tr>
<tr>
<td>IL-1β 5 ng/ml</td>
<td>45.5 ± 3.2</td>
<td>12.3 ± 3.2</td>
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</table>

Conclusion. Regenerating hepatocytes displayed enhanced sensitivity to the growth inhibiting action of IL-1. Consistent with our previous observations of IL-1 secretion by regenerating non-parenchymal cells and the modulation of IL-1 message during liver regeneration these data suggest IL-1 has an important role as a negative regulator of hepatocyte proliferation.
T181

ISOLATION AND CHARACTERISATION OF DNA FROM BLOOD PLASMA USING SENSITIVE MOLECULAR BIOLOGICAL TECHNIQUES. HE Mullighan, P Anker, X Chen, J Lytton, L Ainsworth, A Ballinger, S Carney, EMA Alstead, MJG Farthing. Digestive Diseases Research Centre, St Bartholomew's and Royal London School of Medicine and Dentistry, London, U.K. and Département de Biochimie et de Physiologie Végétale, Université de Genève, Switzerland.

Malignant cells have previously been isolated from the faeces, urine, sputum and pancreatic juice of cancer patients. DNA has also been isolated from blood plasma using laborious and time consuming procedures. This plasma DNA can then be characterised using complex polymerase chain reaction (PCR) assays with subsequent product sequencing. We have developed a simple technique for extracting DNA from blood plasma and have optimised a sensitive PCR assay to detect codon 12 K-ras mutations in plasma DNA.

DNA was extracted from plasma using a two-stage technique. After initial centrifugation of blood, plasma was subjected to high speed microcentrifugation using a commercial kit with in-house modifications. A sensitive PCR assay using sequence specific primers (PASA) was developed to detect K-ras mutations. PASA-PCR results were confirmed by restriction fragment length polymorphism (RFLP) PCR with product sequencing.

The technique developed to extract plasma DNA proved robust and reproducible. Usable quantities of DNA were extracted from 3 ml of plasma obtained from each of 14 pancreatic cancer patients. The optimised PASA-PCR assay was capable of detecting one mutant K-ras gene (TGT, GTT, GCT or GAT) in 10,000 wild-type (GGT) copies. Using the DNA extraction technique and PASA-PCR assay, K-ras mutations were detected in the plasma of pancreatic cancer patients. In cases where paired plasma and tumour samples were available, mutations were identical in both plasma and tumour DNA.

We conclude that simple procedures may be used to extract DNA from the plasma of pancreatic cancer patients. In addition, the PASA-PCR assay may prove useful for detecting minute quantities of mutant K-ras genes within a large excess of wild-type copies.

T182

BASIC FIBROBLAST GROWTH FACTOR PROMOTES PROLIFERATION OF HUMAN GASTRIC ENDOTHELIAL CELLS MA Hull, JL Brough, CJ Hawkey. Division of Gastroenterology, University Hospital, Nottingham, UK.

Exogenous basic fibroblast growth factor (bFGF) promotes angiogenesis and healing of acetic acid-induced gastric ulcers in rats. However the role of endogenously produced FGF in gastric ulcer healing in humans is not well understood.

Angiogenesis in granulation tissue of non-steroidal anti-inflammatory drug (NSAID)-associated gastric ulcers is impaired. Therefore we tested the proliferative response of human gastric endothelial (HuGE) cells to gastric and recombinant bFGF in vitro and without indomethacin.

METHODS Gastric mucosal bFGF was obtained by heparin-separation affinity chromatography. Basic FGF was identified using an ELISA and confirmed by western blot analysis. Primary cultures of HuGE cells were obtained from gastric mucosa of organ donor stomachs and endoscopic biopsies by immunomagnetic selection using anti-PECAM-1 antibody-coated Dynabeads (Dynal, UK). Cultures were grown on 1% gelatin in medium 199 + 30% FCS + 40 µg/ml ECGS + 90 µg/ml heparin. The proliferative response of HuGE cells to gastric and recombinant human FGF (R&D Systems) at 24 hours in the presence or absence of 10^-7 M indomethacin was measured using a MTS assay. Production of 6-keto prostaglandin F1 alpha (PGF1a) by HuGE cells over 24 hours was measured by ELISA (Amersham).

RESULTS Pure gastric FGF eluted from heparin-Sepharose between 2.0 and 2.5 M NaCl. Gastric FGF was estimated to be 19 Kd in size. Gastric bFGF inhibited HuGE cell proliferation (1.5 ± 0.1% control) which was neutralized by an anti-bFGF antibody (R&D Systems). Human recombinant bFGF promoted proliferation of HuGE cells (ED50 5ng/ml). 6-keto PGF1alpha production by HuGE cells was very low (72 pg/ml at 24 hours) and was not stimulated by bFGF. Indomethacin did not impair HuGE cell proliferation in response to gastric or recombinant bFGF.

CONCLUSION Gastric bFGF promotes proliferation of HuGE cells in vitro. Indomethacin did not inhibit bFGF-induced HuGE cell proliferation. NSAIDs may impair gastric ulcer angiogenesis by mechanisms not involving bFGF-induced endothelial cell proliferation.

T183

INHIBITION OF PLASMIN OR UROKINASE PREVENTS BASEMENT MEMBRANE DEGRADATION BY GASTRIC AND OEOSPHELAGEAL CANCER CELLS DF Hewin, T Lai, MN Vipond, D Alderson. Department of Surgery, Bristol Royal Infirmary, Bristol BS2 8HW

Activation of plasminogen by urokinase is thought to be a key step in the initiation of proteolytic degradation of basement membranes to allow tumour cell invasion. The prevention of this process in human oesophageal and gastric carcinoma cell lines was investigated using an in vitro invasion model.

Isotope-labelled subendothelial basement membranes were prepared by incubating human umbilical vein endothelial cells in the presence of [125I]urease (5µCi/ml). Two oesophageal (KYSE 30, KYSE 190) and three gastric (AGS, KATO-III, HGC-27) carcinoma cell lines were seeded onto the membranes and grown in the presence of plasminogen (4µg/ml) plus a plasmin inhibitor, aprotinin (100 IU/ml), or a urokinase inhibitor, amiloride (1mM). Basement membrane degradation was determined after 24 hours by the measurement of activity released into the supernatant. (Results are shown as median counts per minute.)

<table>
<thead>
<tr>
<th>Plasminogen alone</th>
<th>+ Aprotinin</th>
<th>+ Amloride</th>
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<tbody>
<tr>
<td>controls</td>
<td>603</td>
<td>490</td>
</tr>
<tr>
<td>KYSE 30</td>
<td>5102</td>
<td>1109*</td>
</tr>
<tr>
<td>KYSE 190</td>
<td>5070</td>
<td>1126*</td>
</tr>
<tr>
<td>AGS</td>
<td>4852</td>
<td>747*</td>
</tr>
<tr>
<td>KATO-III</td>
<td>4558</td>
<td>651*</td>
</tr>
<tr>
<td>HGC-27</td>
<td>5595</td>
<td>911*</td>
</tr>
</tbody>
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* p<0.05 (Mann-Whitney test) - cells with inhibitor vs. cells without

Basement membrane degradation was significantly reduced by plasminogen inhibition in all cell lines and by urokinase inhibition in five out of six cell lines. These data provide evidence for a mechanism of plasminogen activation by urokinase in upper gastrointestinal tract tumours which may be inhibited to prevent basement membrane degradation.

Endoscopy and biliary T184–T192

RISK OF ASYMPTOMATIC AND SYMPTOMATIC GALLSTONES IN MODERATELY OBESE WOMEN. A LINGUINAL FOLLOW-UP M Acaroglu, D Blondona, M Pascu, A Georgoanea, R Badescu, M Prelieic. 3rd Medical Clinic, University of Medicine & Pharmacy, Cluj-Napoca, Romania

Gallstone (GS) incidence was analyzed in 157 moderately obese women (BMI 31.4+6.5 kg/m2) followed up by ultrasound for 2-6 yrs (mean 3.9 yrs). Patients having diseases with lithogenic risk were not included in the study. All the enrolled women had normal cholecsystosonogram at the beginning of the study. Age, family history of GS or obesity, parity, age of obesity onset, HLP type, plasma cholesterol(total,HDL, LDL), and triglycerides were assessed. The Student t, the Mann-Whitney rank sum and the Fisher exact tests were used, as well as the multiple logistic regression for the multivariate analysis.

During the survey, 16 of 157 women (10.2%) developed GS. GS were asymptomatic in 12 persons (75%). The cumulative incidence of GS was 2.58/100 person yrs. The following risk factors were associated with GS formation as independent variables: age (p=0.0059), family history of GS (p=0.0094), early obesity onset (p=0.0104), HLP IV (p=0.003) and triglyceridaemia (p=0.0008).

Obesity is a well documented lithogenic factor in obese women. The magnitude of the increased risk and the rates of GS occurrence, however, have not been well quantified, except for one study on the risk of symptomatic GS (1). Our study estimated the incidence of both asymptomatic and symptomatic GS, and during the follow-up, most of the detected GS were asymptomatic. This explains the higher GS incidence rate found. The present study has also shown that the chances of an obese woman having GS are higher with a family history of GS; an early obesity onset, a type IV HLP and increasing age. A high risk class might be thus identified among obese women, offering a more realistic approach for the primary prophylaxis of GS.