Cardiac specific alpha-myosin antibodies in alcoholic liver disease: An immunological role in asymptomatic left ventricular enlargement?

AC Douds, MK Baig, C Page, M Yamada, JG Goldman, JD Maxwell, W McMenna. St. George's Hospital Medical School, London SW17 0RE

Asymptomatic left ventricular enlargement (LVE) occurs in up to 30% of patients with alcoholic liver disease (ALD). The presence of IgG antibodies to cardiac acetylcholinesterase in alcohols suggests 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 normals (age 56 ± 7.6, 70 males), and in 203 normals (age 45 ± 16, 100 males) using an ELISA. Patients with ALD also had 2D echocardiography and an SAECG.

Results Anti-IgG myosin antibodies were present in 9/51 (18%) patients with ALD (P<0.0001, χ2, 4/92) (4/92) 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 rates 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 that 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.

Increased expression of circulating leucocyte adhesion molecules CD11b and CD18 in inflammatory bowel disease.

CE Collins, C Davies, MO Macey*, DA McCarthy*, DS Rampion. 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. Among 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 sulphasalazine 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 CD18 in unfixed 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 (MFI), 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>CD11b</th>
<th>GNLs</th>
<th>MNs</th>
<th>GNLs</th>
<th>MNs</th>
</tr>
</thead>
<tbody>
<tr>
<td>healthy controls</td>
<td>30 (26-34)</td>
<td>27 (22-38)</td>
<td>40 (39-41)</td>
<td>86 (82-98)</td>
</tr>
<tr>
<td>active CD</td>
<td>10</td>
<td>97 (55-116)</td>
<td>106 (92-121)</td>
<td>571 (53-108)</td>
</tr>
<tr>
<td>inactive CD</td>
<td>11</td>
<td>71 (52-115)</td>
<td>731 (90-260)</td>
<td>47 (65-82)</td>
</tr>
<tr>
<td>active UC</td>
<td>11</td>
<td>69 (35-100)</td>
<td>811 (65-112)</td>
<td>62* (48-101)</td>
</tr>
<tr>
<td>inactive UC</td>
<td>9</td>
<td>85* (43-116)</td>
<td>84 (68-132)</td>
<td>46* (52-62)</td>
</tr>
</tbody>
</table>

*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.
INCREDIBLE INDUCTIVE CYCLOOXYGENASE ASSOCIATED
WITH TREATMENT FAILURE IN ULCERATIVE COLITIS
Divs Gastroenterology, Histopathology & Immunology, University Hospital, Nottingham NG7 2UH, & Lincoln County Hospital.

INTRODUCTION: The cyclooxynsas (COX)-2 gene is induced at sites of inflammation and in colonic carcinoma and normally suppressed by steroids. Since COX-2 could be important for malignant change or prostaglandin dependent secretion in ulcerative colitis, we investigated COX-2 expression in colonic mucosal samples from patients with ulcerative colitis, and normal controls, and related results to expression of inducible nitric oxide synthase (iNOS) and interleukin-1ß (IL-ß) since these are normally co-induced.

METHODS: Mucosal biopsy samples were obtained from 17 patients with ulcerative colitis (8 surgery, failure steroid), 9 newly diagnosed, and 6 normal controls. iNOS and interleukin-1ß (IL-ß) were determined by enzyme linked immunosorbent assay (ELISA) and reversetranscription polymerase chain reaction (RT-PCR) amplification of cDNA sequences for COX-2 (323 base pairs, 23 cycles), iNOS (453 base pairs, 28 cycles), and IL-ß (220 base pairs, 25 cycles) and GAPDH (reference housekeeping gene, 650 base pairs, 23 cycles) was ran'd out and PCR product assayed using a semi quantitative enzyme linked immunosorbent assay. RESULTS: PCR product levels (mean+ standard deviation) expressed as proportion of GAPDH x 100 for COX-2 are shown in the table.

COX-2     IL-ß     iNOS
Controls (n=23) 66(13) 1.7 1.4
Ulcerative colitis (n=17) 7(3-4) 1.8 3.8

P(Mann Whitney) 0.016 <0.001 <0.05

Sub group analysis showed COX-2 PCR product levels were significantly elevated in patients having surgery for failed medical treatment (13.3-30) vs 10 (4-15), p=0.004, but not in newly diagnosed untreated patients (10 (7-34) vs 7 (5-13), p=0.37). COX-2 levels correlated with acute inflammation and IL-ß (RS=0.43, p=0.008) but not iNOS (p=0.107). In conclusion, COX-2 expression is increased in active ulcerative colitis. A sub group of patients, in whom medical treatment fails, may be resistant of the suppressive effects of steroids.

PROGNOSTIC VALUE OF WHOLE GUT LAVAGE FLUID ANALYSIS IN CROHN'S DISEASE.
S Ghosh, Sabrina Accuff, Anne Ferguson. Gastrointestinal Laboratory, Department of Medicine, University of Edinburgh, Western General Hospital, Edinburgh EH4 2UQ.

There are no reliable methods to determine the probability of relapse in Crohn's disease. Though prognostic indices based on ESR, CRP, albumin and a-glycoprotein have been designed to predict outcome, serum proteins poorly reflect mucosal immune events, and such indices have not generally been found useful. Analysis of whole gut lavage fluid (WGLF) is an objective measure of activity of IBD, and gut immune events such as neopterin migration and proinflammatory cytokine production can be investigated. The aim of this study was to investigate whether WGLF parameters are of prognostic relevance with respect to relapses of disease. Thirty patients with Crohn's disease were in remission (no symptoms, normal ESR, CRP, white cell count, WGLF IgG < 10µg/ml) at the time of whole gut lavage with polyethylene-glycol electrolyte (Klean-Prep) solution. Granulocyte elastase (GE), a marker of neutrophils in WGLF was assessed using a highly specific substrate, L-tyrosylamyl-L-prolyl-L-valine-p-nitroanilide (Quadratex, UK). IL-ß in WGLF was assessed using a commercial ELISA kit (Gisthor, USA). Twelve patients had detectable GE in WGLF, and 10 of them were not in remission at 1 year after the initial lavage; 4 had a relapsing course (needing steroid therapy), 5 were steroid dependent and 1 was on immunosuppressive therapy. In contrast, 18 patients in remission had undetectable GE in WGLF, and 12 of them were in remission at 1 year needing no therapeutic intervention (p < 0.01). Ten of the patients in remission had detectable IL-ß in WGLF and 8 of them were not in remission at 1 year after the initial lavage; 3 had a relapsing course (needing steroid therapy), 4 were steroid dependent and 1 was on immunosuppressive therapy. In contrast, 12 patients had detectable IL-ß in WGLF, and 12 of them were in remission at 1 year needing no therapeutic intervention (p < 0.01).

In conclusion, in Crohn's disease patients in remission, detectable GE or IL-ß in WGLF is associated with a poorer outcome at 1 year.

IMAGING OF E-SELECTIN, A SPECIFIC MARKER OF ENDOTHELIAL CELL ACTIVATION, IN THE EVALUATION OF INFLAMMATORY BOWEL DISEASE.
MA Blaas, PT Chapman, AM Peters, DO Haskard, HJF Hodgson.
Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London W12 ONN

E-Selectin expression is up-regulated in response to pro-inflammatory cytokines and makes an integral part of the process leading to accumulation of inflammatory cells in the tissue. Tissue E-Selectin expression is increased in inflammatory bowel disease (IBD) and we have investigated its potential for targeting the inflammatory activity in IBD.

METHODS: 9 Ulcerative colitis (UC) and 7 Crohn's disease (CD) patients with varying degrees of active inflammation were selected with prior consent. Following intravenous administration of 111-Indium (111-In) labelled antibody to E-Selectin, anterior, posterior and lateral images were obtained 4hrs, 24hrs and 48hrs post injection by gamma camera equipped with medium-energy-general-purpose collimator. A 99-Technetium (99Tc) labelled scan was performed the following day.

RESULTS: 9 out of 16 patients had active areas of inflammation, defined by 99Tc labelled white cell scans. The extent of inflammation demonstrated by 111-In labelled E-Selectin scan ranged from pan-colitis in active UC to localised areas of inflammation both in UC and CD. 10 patients with active IBD had positive E-Selectin scans. The results were concordant in 9 and discordant in 3. Four patients were negative on both types of scan.

111-In labelled E-Selectin antibody images correlated well with the extent and activity of the disease, however the correlation with clinical indices of the level of severity of the disease was less significant.

CONCLUSIONS: We have demonstrated that E-Selectin scan can localise the area of inflammation through the process which is simpler than white cell scan. It is more versatile and can be done in neutropenic patients.

IN SITU IMMUNE RESPONSES IN CROHN'S DISEASE: A COMPARISON WITH ACUTE AND PERSISTENT MEASLES VIRUS INFECTION.
AJ Wakefield, R Sim, A Akbar, R.E.Pounder, A.P.Dhillon. Inflammatory Bowel Disease Study Group and Department of Immunology, Royal Free Hospital School of Medicine, Rowland Hill Street, Hampstead, London NW3 2PF, UK.

The implied aetiological association of measles virus with Crohn's disease would be supported by detection of an immune response to infected cells in affected tissues. This study sought to detect and characterise in situ immune responses to measles virus in both acute and persistently infected tissues, and in particular, Crohn's granuloma. Serial tissue sections from cases of Crohn's disease (n = 17), tuberculosis (n = 9), acute intestinal ischaemia (n = 5), acute measles pneumonia (n = 2), acute megalocytes appendicitis (n = 1), subacute sclerosing panencephalitis (SSPE; n = 1), and measles inclusion body encephalitis (MIBE; n = 1), were examined. Single and double immunohistochemical labelling was performed to identify both cytoxoyc lymphocytes (CD8, CD4, perforin, Leu 7, CD45RO, CD45RA) and macrophages (KP1). The relationship of these cells to measles infected cells was identified by double immunolabelling with anti-measles virus nucleoprotein antibody. In both acute measles appendicitis and SSPE, CD8/TIA/-cytotoxic lymphocytes (CTL) targeted infected cells. In the other tissues that were positive for measles virus including Crohn's disease (13/17) - where staining was largely confined to granuloma, MIBE, fatal pneumonitis, and 1 tuberculous granuloma, infected cells appeared to be targeted by macrophages rather CTL. The CTL in Crohn's granulomata were Leu 7+ and perforin /CD45RO (naive). CTL in both tuberculous and Crohn's granulomata were similar in their peripheral distribution, number and phenotype. The data suggest that measles-specific CTL responses may be attenuated in Crohn's disease compared with acute measles appendicitis and SSPE, and secondly, that an abnormal macrophage response to persistent measles virus infection of the intestine may result in granulomatous inflammation.
DOSE LOADING WITH ORAL MESALAZINE: OPTIMISING DRUG CONCENTRATIONS IN THE MUCOSA F. Husain, R. Ajan, N. Trudgill, S. Riley. Dept of Gastroenterology, Northern General Hospital, Sheffield, U.K.

Mesalazine based products are widely used in the maintenance of ulcerative colitis remission. However, the optimal dose remains unknown since dose-ranging studies have yielded conflicting results.

We have therefore studied steady-state kinetics and mucosal drug levels in healthy volunteers taking progressively larger doses of oral mesalazine. 12 subjects (7 male, aged 18-30), were given delayed release mesalazine 400mg tds for 7 days. Serial blood and rectal mucosal samples and urine and stool were collected over 24 hours. Following a drug free interval the same subjects repeated the protocol with 800mg tds and 1600mg tds. Samples were analysed for 5-aminosalicylic acid (SASA) and N-acyetyl-5-ASA (NASASA) by HPLC. Median results are shown below (NASASA/NASASA).

<table>
<thead>
<tr>
<th>Plasma/Auc</th>
<th>Urine Faecal</th>
<th>Mucosa/Auc</th>
</tr>
</thead>
<tbody>
<tr>
<td>(ug/ml)</td>
<td>(mg)</td>
<td>(mg/hr/ug)</td>
</tr>
<tr>
<td>400mg</td>
<td>17.2±2.3</td>
<td>249±8.1</td>
</tr>
<tr>
<td>800mg</td>
<td>30.9±15.4</td>
<td>523±7.85</td>
</tr>
<tr>
<td>1600mg</td>
<td>56.8±4/6</td>
<td>1468±44/6</td>
</tr>
</tbody>
</table>

AUC (area undercurve).

Following serial dose-doubling with oral mesalazine: i) Combined faecal and urinary excretion (SASA+NASASA) increase progressively. ii) Urinary excretion (SASA+NASASA) progressively exceeds faecal excretion. iii) The proportion of 5ASA to NASASA increases indicating saturation in urine, plasma, stool and rectal mucoea. iv) At higher dose, the increase in rectal mucosal levels is modest in comparison with the increase in plasma concentration and urinary excretion.

PSYCHOLOGICAL MORBIDITY IN INFLAMMATORY BOWEL DISEASE: THE IMPACT OF A COUNSELLING SERVICE

Smith, G., Leman W, Roger D, Palmer KR. Gastrointestinal Unit, Western General Hospital, Edinburgh.

It is often assumed that counselling may alleviate many of the psychological problems associated with Crohn's disease (CD) and ulcerative colitis (UC) although this has not been proven.

Fifty patients with CD (38 females, median age 38), 50 UC patients (22 females, median age 38) and a group of 50 healthy volunteers (HV,27 females, median age 34) underwent structured interviews and completed a range of questionnaires measuring several facets of psychological wellbeing (Hospital Anxiety and Depression Score (HAD), Attitudes & Preferences (AP) and Styles & Strategies (SS) questionnaire). Patients with CD and UC were then randomised to receive either a counselling package or routine clinical follow-up. The counselling package consisted of disease specific educational videos, information booklets and the teaching of stress management techniques. Patients were reassessed at six months.

At baseline, the scores for HAD, SS and AP were within the normal range in patients with UC and HV. CD patients had higher anxiety level than HV (mean anxiety score CD 10.1, UC 7.7, HV 6.8, p<0.001). CD patients also demonstrated significant maladaptive coping mechanism on SS score (mean maladaptive score CD 19.1, UC 15, HV 12, p<0.001). At follow-up, the anxiety score of counselled CD patients improved significantly (p=0.005) as did their maladaptive coping mechanism (p<0.001).

Psychological morbidity is common in CD and can be quantified using validated questionnaires. These aspects of psychological morbidity can be effectively treated by specific counselling.

A COMPARATIVE STUDY TO INVESTIGATE FACTORS ASSOCIATED WITH IRREGULARITIES OF BOWEL FUNCTION AMONG HEALTHY BENGALEES IN CALCUTTA AND SHEFFIELD AND ENGLISH SUBJECTS IN SHEFFIELD. S. SENGUPTA, N.W.READ CENTRE FOR HUMAN NUTRITION, NORTHERN GENERAL HOSPITAL, SHEFFIELD S5 7AU, U.K.

INTRODUCTION Factors underlying irregular bowel habits (IBH) are unknown but previous studies have implicated gender and diet. These may vary in different cultural settings and in the UK, it has been suggested that more females than males (2:1) have Irritable bowel syndrome (IBS) while in India, it seems that more males than females (3:1) have IBH. This study investigates the incidence of IBH in healthy general population from India and UK and explores how patterns of bowel habit are related to different cultural background, gender, psychological factors and patterns of eating and other behaviours.

METHODS A questionnaire on bowel habits, eating behaviour, mood and other behaviours was completed by 318 Bengalis in Calcutta (156 male and 159 F) and 108 English subjects (51 M and 57 F) and 233 English in Sheffield (101 M and 132 F).

RESULTS 52.7% of Bengalis in Calcutta reported IBH compared with 43.4% Bengalis and 49.8% English in Sheffield. IBH was more common in female English subjects in UK (m:f; 39:77) and in male Bengalis in Sheffield (m:f; 32:17) and Calcutta (m:f; 97:69). A greater percentage of Bengalis and English with IBH than those with regular bowel habits (RBH) reported inconsistent meal timings, frequent snacking, breakfast skipping, frequent micrturition, and sleep disturbance. None of these could account for the genetic specificity of bowel habit within the three populations. Other factors were common in Bengalis with IBH which could account for male preponderance were consumption of tea, coffee, alcohol and battled leaf, and psychological status. 72% of male Bengalis in Calcutta and 63% of male Bengalis in Sheffield with IBH were married compared with only 44% Bengali males in Calcutta and 26% Bengali males in Sheffield with RBH. Frequent mood change (FMC) was also more common in IBH subjects from all three groups and showed a female preponderance among the English subjects and a male preponderance among the Bengalis in Sheffield and Calcutta.

CONCLUSION An association between IBH and irregularities of several different behaviours and mood was observed in all three groups, though the data suggest that marriage status and alcohol consumption and FMC are all associated with the male preponderance within the Bengalis with IBH.
**Small intestine and diarrhoea**

F239–F247


**Background.** Histologically, administration of indomethacin (indo) causes villus shortening, eosinophil infiltration and microvascular distortion, prior to ulceration. These changes may compromise blood flow and lead to mucosal necrosis.

**Aim.** This hypothesis was tested by correlating histological changes with changes in villus blood flow, measured in vivo using 14C-doses of indo.

**Methods.** In two groups of rats (indo vs. vehicle), ileal indo (15 mg/Kg) was given at 4 or 6 h preoperatively. Luminal and plasma levels were measured in order to determine the optimum dosing for subsequent experiments. After anaesthesia, exteriorised villi were observed by fluorescent microscopy using iv FITC dextran and labelled red cells. Blood flow in surface arcade vessels was calculated from measurements of velocities and diameters.

**Results.** Oral indo resulted in peak luminal and plasma indo concentrations of 100 mg/ml. In vivo observation revealed groups of villi with vascular stasis. Histologically the villi were shortened, and had distorted epithelium and vessels. Combined topical and iv indo, given pre-operatively, induced immediate progressive slowing of blood flow in individual villi, and complete stasis within 15-45 mins in individual villi in all animals (Fig). Blood flow in adjacent villi was normal. At this time point, histology showed groups of shortened villi with distortion (but no loss) of surface epithelium overlying focci of vascular stasis, and normal surrounding villi. In both lesion groups, stasis occurred only on the mesenteric border between vasa-recta. All control animals had normal blood flow (2.6 ± 0.1 1/mm) for 1.5 hrs.

**Conclusions.** Oral dosing with indo, or combined topical and iv dosing at an ulcerogenic level, produces pre-ulcerative villus shortening, associated with severe focal interference of capillary blood flow. Thus focal ischaemic changes are likely to be involved in induction of lesions.

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**DETECTION OF FAECAL INCONTINENT EPISODES USING A NEW AMBULATORY WETNESS DETECTOR**

J. Gunn, R. Farouk, J.R.T. Monson, G.S. Duttie; University of Hull, Academic Surgical Unit, Castle Hill Hospital, Cottingham, North Hamberside.

**Internal anal sphincter ambulatory assessment has suggested the anorectal pressure gradient may be reversed during faecal soiling. However patients are commonly unable to perceive these events. The aim of this study was to develop a wetness detector that would allow us to identify objectively episodes of faecal incontinence.**

The wetness detector was constructed from a 5mm catheter with two copper electrodes set 3cm apart. A low voltage AC current was passed down the catheter and when the moisture levels increased, then electrical resistance between the two electrodes would reduce allowing an increase in conductivity indicating faecal incontinence. Simultaneous analrectal manometry was performed to correlate these events with changes in the anorectal pressure gradient.

10 patients (median age 58, range 44-76) with faecal incontinence were assessed for a median of 8 (4-18) hours. Episodes of faecal leakage were detected in all patients characterised by a rise in conductivity. A positive recto-anal pressure gradient was recorded for all of these events.

In conclusion, a sensor has been developed which can accurately and objectively detect episodes of faecal incontinence. The ability to detect occult episodes of faecal leakage has clear implications for the assessment of patients complaining of faecal incontinence.

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**SERUM SUCROSE: A NEW SCREENING TEST FOR COELIAC DISEASE.**

M.A. Cox, T.H. Iqbal, K.O. Lewis, B.T. Cooper. Gastroenterology Unit, City Hospital, Dudley Road, Birmingham. B18 7QH.

An accurate non invasive test to screen for coeliac disease (CD) would be popular with patients and reduce the need for small bowel biopsy. Until recently such tests were inaccurate. IgA endomysial antibodies have been found to have a high sensitivity and specificity for CD. Small intestinal permeability tests involving urinary recovery of ingested markers are highly sensitive but not specific for CD. During the development of techniques to measure permeability in serum, an unidentified peak was found in the serum from untreated coeliac patients but not in other sera. This peak was later found to be sucrose. The aims of this pilot study were to determine if serum sucrose is a marker for untreated CD and to compare the results with endomysial antibody titres. 20 consecutive newly diagnosed coeliacs, 15 celiacs on a strict gluten free diet for more than 12 months and 15 healthy normal controls were studied. All were given a drink containing approximately 8g of sucrose and had blood taken 30-45 minutes later: serum was deproteinised using the method of Somogyi and analysed using HPLC and pulsed amperometric detection for the presence or absence of sucrose. Using the Ass Clin Biochem 1995 AER2:89-90). Sucrose was present in all 20 untreated coeliacs but in none of the treated coeliacs or normal controls. 17 untreated coeliacs were endomysial antibody positive; all the treated coeliacs and normal controls were negative. Serum sucrose showed promise as an indirect marker of villous atrophy and coeliac disease.