International Inflammatory Bowel Disease

The 30 abstracts of presentations at the International Inflammatory Bowel Disease Meeting, held in Chester on 15–16 April 1996 are printed below.

Isolation of human intestinal microvascular endothelial cells from normal and inflamed gut: expression of MadCAM-1

J WILSON, R THOMPSON, M RHOADES, S L BLOOM, R THOMPSON, M RHOADES, S L BLOOM (Department of Colorectal Surgery and Cellular Pathology, John Radcliffe Hospital, Oxford) The anal transitional zone (ATZ) has been defined by Fenger as the zone interposed between uninterrupted colorectal type mucosa above and uninterrupted squamous epithelium below. He described mapping the ATZ and quantified it by whole specimen staining of the anus with alcin blue (AB).

This paper describes a new technique for mapping the ATZ by computer image processing of the microscopy for multiple longitudinal sections through the anus at 3 mm intervals. Fifteen areas have been studied by this and the AB technique. Computer maps and photographs of specimens stained with AB were assessed by computer image analysis measuring the areas and positions of the epithelial components, ATZ, dentate line, and the lower border of the internal sphincter.

The Table shows mean values of various measurements (units = cm or cm²).

<table>
<thead>
<tr>
<th>AB technique</th>
<th>Computer map</th>
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<tbody>
<tr>
<td>TE area</td>
<td>3.42</td>
</tr>
<tr>
<td>TZ area</td>
<td>3.04</td>
</tr>
<tr>
<td>TZ span</td>
<td>1.12</td>
</tr>
<tr>
<td>DL height</td>
<td>1.00</td>
</tr>
<tr>
<td>Upper border TZ</td>
<td>1.45</td>
</tr>
<tr>
<td>Lower border TZ</td>
<td>0.77</td>
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</table>

Columnars in the ATZ were infrequent, median = 0 (range 0–16), mean area per ATZ = 0.08 cm². The AB technique overestimated the amount of transitional epithelium by 2–5 times, in comparison to the histology, by significant staining of the nuclei of non-keratinised simple squamous cells.

On the basis of this work there seems little concern about retaining the ATZ in restorative proctocolectomy and attention should be focused on the columnar cuff of mucosa in the anal canal between the ponsch anal anastomosis and the upper border of the ATZ.

5-Amino-salicylic acid (5-ASA) induced nephrotoxicity is dose dependent in rats

C LUNDBERG, I ÅSBERG, G CARLIN, T BERGLINHOJ (Department of Pharmacology, Pharmacia AB, Uppsala, Sweden) Nephrotoxicity has been reported in IBD patients receiving mesalazine therapy probably caused by too high and variable systemic exposure of 5-ASA. The purpose of this study was to investigate whether 5-ASA induced nephrotoxicity is 5-ASA dose dependent or a hyper-sensitivity reaction in the rat.

Saline, 5-ASA (100, 300, 600, and 900 mg/kg), or acetyl (Ac)-5-ASA (900 mg/kg) was injected intraperitoneally daily for 3 days in rats. Twenty four hour urine samples were collected and analysed for micro albumin (glomerular damage), N-acetyl-D-glucosaminidase (NAG), and alkaline phosphatase (ALP) (tubular damage), and osmolality as measures of nephrotoxicity.

The two lower 5-ASA doses and Ac-5-ASA did not affect any of the variables measured. However, in rats receiving 600 and 900 mg 5-ASA/kg, respectively, increased urine NAG and albumin levels and decreased osmolality were observed.

We have shown that even a short systemic load of 5-ASA, in contrast with Ac-5-ASA, in rats causes a renal glomerular and tubular damage that is dose dependent. These results suggest that mesalazine formulations that cause a high systemic exposure of 5-ASA in IBD patients, may have a potential risk of being nephrotoxic.

Is therapy always necessary for patients with ulcerative colitis in remission?

G BIANCHI FORBO (Gastrointestinal Unit, 'L Sacco' Hospital, Milan, Italy) The aims of this study were (1) to compare, in a double blind, double dummy, randomised fashion, the efficacy of a delayed release 5-ASA (Asacol 1 g/d) versus placebo (PI), for a follow up period of one year, in 112 patients (66 M, 46 F, mean age 35), with intermittent chronic UC in clinical and endoscopic remission with salicylates for at least one year, and (2) to verify if duration of disease remission affects the relapse rate. Clinical, endoscopic, and histological findings were assessed every 6 months. Assuming that a minor duration of remission may be associated to higher relapsing risk, the patients were stratified according to the length of their disease remission, in groups (A) (5-ASA 26, PI 35, in remission from 12 to 24 months) and (B) (5-ASA 28, PI 23, in remission over 2 years, median 4 years). 'End point' of the study was considered the finding of clinical and endoscopic relapse. A Kaplan-Meier life table analysis was used to calculate the relapse rate. Fifty four patients were treated with 5-ASA and 58 with PI. The relapse rate was similar in both groups after 6 months (5-ASA 8 or 54 (15%), PI 14 of 58 (24%), p=0.15, IC95 0.23+0.05), while a statistically significant difference was found after 12 months of therapy (5-ASA 11 of 54 (20%), PI 23 of 58 (40%), p=0.016, IC95 0.35–0.02). 5-ASA was significantly more effective than PI in preventing relapse at 12 months in group A (5-ASA 6 of 26 (23%), PI 17 of 35 (49%), p=0.037, IC 95
Differential display polymerase chain reaction based isolation of colitis related genes from mice with experimental colitis

APPSANI S, KRISHNARAO, GYORGY FREUND (Centre for Experimental Therapeutics and Reperfusion Injury, Department of Anesthesia, Brigham and Women’s Hospital and Harvard Medical School, Boston, MA, USA). The DD-PCR method was used to identify more than 100 partial cDNA fragments that showed significant changes in gene expression between normal and diseased colon at mRNA level. These partial cDNAs were cloned, sequenced, and characterised to conform their disease specificity. Sequence comparison with Genebank revealed that 60% of our isolated cDNAs represented new, previously unknown mouse genes. Twenty per cent of them showed strong homology with known mouse sequences including mitochondrial, nuclear, and ribosomal genes. We also found that 10% of cDNAs, like IL-1a, IL-1b, tryptophan, TRC alpha/delta, nicotinic ACh receptor, and ras recision genes were expressed at high levels in colitis. We conclude that our DD-PCR approach has a remarkably high probability of isolating novel colitis related mouse genes. These cDNA fragments were used as molecular probes on northern blots to further document their involvement in colitis. Funded by Crohn’s and Colitis Foundation of America.

Image endothelial cell activation: a new imaging system using In-111 labelled F(ab')2 fragment, monoclonal antibody E-selectin in the assessment and diagnosis of inflammatory bowel disease activity

M A BHATTI, P T CHAPMAN, A M PETERS, D O HASKARD, H J F HODGSON (Royal Postgraduate Medical School, Hammersmith Hospital, London) Expression of E selectin, an endothelial cell specific adhesion molecule, is increased both in ulcerative colitis (UC) and Crohn’s disease (CD). E selectin is induced by pro-inflammatory cytokines and contributes to the accumulation of inflammatory cells in tissues, which makes it an ideal target for imaging the inflammatory activity in inflammatory bowel disease (IBD). Sixteen patients with clinically active IBD were sequentially imaged with In-111 anti-E selectin F(ab')2 fragment and 24 hours later with Tc-99m leucocytes. Nine of 16 patients had areas of active inflammation determined by leucocyte scan while 10 of 16 patients had positive E selectin scan. Positive scans were concordant in 9 and discordant in 3. Four patients were negative with both. The extent of inflammation demonstrated by E-selectin scan ranged from pancolitis in UC to localised areas of inflammation in both UC and CD. In-111 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 disease was less significant.

We have demonstrated that E selectin scan can localise the areas of inflammation through the process, which is simpler than whole cell scan. Anti-endothelial scintigraphy is more versatile to use, applicable to neutrophenic patients, and merits further development.

Heparin therapy in refractory ulcerative colitis – an update

P R GAFFNEY, A GAFFNEY (Department of Surgery, Mallow General Hospital, Mallow, Co Cork, Ireland). At the meeting of the AGA we reported a beneficial effect associated with heparin therapy in nine of 10 cases with refractory UC. As a number of initially promising treatments for IBD have subsequently proved disappointing, we decided to review (a) the outcome of the patients in our pilot study, (b) unpublished case reports of further patients treated with heparin, and (c) recent publications on heparin therapy in refractory UC.

Five of the nine patients who went into remission on heparin had a recurrence of the disease; four of these responded to further heparin therapy. Three patients had colonic symptoms (for example, obstruction; pseudopolyps). All three were in clinical remission at the time of surgery. Seven further patients with refractory UC were treated at our hospital. Three had fulminant and four mild disease. All went into remission on intravenous heparin with sulphasalazine, having initially failed to respond on subcutaneous heparin. We received reports of nine patients with Crohn’s disease at centres here and abroad, seven of whom had a favourable response. An open pilot study of heparin in nine cases of refractory UC reports remission in seven cases, with one relapse.

A case study reports the rapid and sustained resolution of pyoderma gangrenosum and refractory UC on intravenous heparin.

We have shown that heparin, used intravenously and with sulphasalazine, appears to be effective in the treatment of refractory UC, and warrants larger controlled trials.

Withdrawal rates because of diarrhoea in 5-ASA treated patients with ulcerative colitis are low when diarrhoea is taken with food and dose titrated

GUNNAR JÅRNEROT (Department of Medicine, Division of Gastroenterology, Örebro Medical Centre Hospital, Örebro, Sweden). Earlier the withdrawal rates because of diarrhoea in 5-ASA treated patients with ulcerative colitis were high. The insight into adaptation and dose dependency prompted me to recommend the drug to be taken after a meal and dose titrated. This appeared to almost eliminate the diarrhoeal problem.

To assess this a review was performed of controlled and reliable uncontrolled studies in the literature (n=22) or available as Pharmacia internal reports (n=2).

<table>
<thead>
<tr>
<th>Administration</th>
<th>Number</th>
<th>Withdrawals (%)</th>
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<tbody>
<tr>
<td>Not titrated or with food</td>
<td>585</td>
<td>45 (9.2)</td>
</tr>
<tr>
<td>Titrated plus/minus food</td>
<td>670</td>
<td>22 (3.3)</td>
</tr>
<tr>
<td>Food plus/minus titration</td>
<td>419</td>
<td>16 (3.8)</td>
</tr>
<tr>
<td>Food plus/minus titration and Enteric-ph coating, titrated with food</td>
<td>454</td>
<td>13 (2.9)</td>
</tr>
</tbody>
</table>

Using 5-ASA, diarrhoea is reduced from 10% to 3% or less. The 5-ASA coated tablets appear to empty after the food giving the same effect as when taken on an empty stomach.

Assessing function following restorative proctocolectomy

S A WAKEFIELD, A J SHORTHOUSE (Department of Surgery, Royal Hallamshire Hospital, Sheffield). Restorative proctocolectomy is a technically demanding operation with problems both in the short and long term. Careful longterm follow up of patients is important to define longterm outcome. A simple, accurate functional scoring system is necessary to compare results between different centres and modifications in the operative technique. The scoring system most commonly used is based on simple, reliable scoring systems which are closely linked. We have modified this scoring system to include only 5 important independent variables: daytime and nocturnal frequency, urgency, daytime and nocturnal soiling. The categories of score due to the two scoring systems has been determined. Over an 8 year period 64 patients have undergone restorative proctocolectomy in our unit (males 40, mean age 35-4, ulcerative colitis 60). Pouch function scores (253 visits) have been collected prospectively at regular intervals following surgery (3-60 months) and scoring system results correlated. A strong correlation between the two systems was found using Spearman’s correlation (r=0.73, p=0.000). This was confirmed using the method of Altman and Bland for parallel scoring systems. An acceptable pouch function score is required to measure and compare performance for research purposes. We believe our modified functional scoring system has several advantages over existing systems. These include fewer independent variables with easier data collection and metric scaling.

Surveillance in ulcerative colitis: a national lottery

S A WAKEFIELD, R ACKROYD, J WILLIAMS, M W REED (Department of Surgery, Royal Hallamshire Hospital, Sheffield). Patients with chronic ulcerative colitis (UC) have an increased risk of colorectal cancer (CRC). Disease duration and high dose corticosteroid use are associated with a higher risk. Surveillance colonoscopy to detect dysplasia or early CRC is often undertaken but there are no national guidelines.

---0.48--0.02--- In contrast, no statistically significant difference was seen between the two treatments, at 12 months of follow up, in group B.

In conclusion, this study shows that 5-ASA prophylaxis is necessary for preventing UC relapses in patients in remission for less than 2 years; on the other hand, it casts doubts as to whether or not continuous maintenance is still necessary in patients with prolonged endoscopic and clinical remission. A prognostic model is in progress to better define the possible subgroups of patients in whom the maintenance treatment could be stopped.
A questionnaire was sent to all 75 clinicians in Trent who have a regular colonscopy list and 56 (75%) responded.

**Pancolitis.** Surveillance colonscopy is undertaken by 49 (87.5%) clinicians with an interval of: 1 year (49-0%), 2 years (34-7%), 3 years (14-2%), and 5 years (2-0%). Surveillance begins between 1 and 12 years from diagnosis with the commonest repeats being 10 years (49-0%) and 8 years (20-4%).

**Left sided colitis.** Only 19 (38-8%) clinicians offer surveillance colonscopy to this group with an interval of: 1 year (21-1%), 2 years (36-2%), 3 years (10-5%), and 5 years (3-5%). Surveillance begins between 1 and 20 years from diagnosis with the commonest repeats being 10 years (26-3%) and 15 years (26-3%).

Tartar epidemiology was commonly 70 or 75 (67-4%) although many clinicians did not have an upper age limit (22-5%). When asked about the need for national surveillance guidelines in patients with UC 49 (87-5%) were in favour with only 1 (2-0%) respondent against. Six (10-7%) gave no reply.

There is wide variation in surveillance of patients with chronic UC and a clear need for national guidelines.

**Ulcerative colitis in the elderly: commoner in men and milder**

G RIEGEL, M TARGTALGIONE, R MARMO, R CARRATU and ‘Gruppo Italiano Studio Colon-Retto – GISC’ (Seconda Universita, Napoli, Italy) Data concerning the natural history of idiopathic ulcerative colitis (UC) in the elderly are generally unreliable, often being based on too small a sample size.

We report data from 1255 consecutive UC patients (59-7%) male; mean age 35 years) who were recruited between 1990 and 1994 in 17 clinics distributed around Italy. The M:F sex ratio was 2:08 in the older (>=65) group of 114 patients and 1:44 in the 141 younger patients.

All 1255 patients were also grouped into quartiles according to age (0-25; 25-35; 35-60; =51 years) and the M:F ratios in these quartiles were 1:08; 1:38; 1:70; 2:00 with a 5-0F:1-0M sex distribution increasing with increasing age and increasing M:F ratio. When patients =51 years were compared with those 0-25 the older patients showed significantly lower disease activity at diagnosis (p<0.03), required corticosteroid requirement (p<0.02), and fewer extraintestinal complications (p<0.02).

In conclusion a male preponderance and milder disease characterise UC in the elderly.

**Prevalence of periodontal and oral mucosal lesions in inflammatory bowel disease**

G RIEGEL, M TARTAGLIONE, M T VIETRI, R CARRATU; **G COLELLA, F DE LUCAS, A LANZA, G P TARTARO (Cattedra di Gastroenterologia, II Universita di Napoli, Italy) Oral mucosal and periodontal lesions are well recognised in ulcerative colitis (UC) and Crohn’s disease (CD) but generally considered to be occasional.1

We have assessed a consecutive series of 96 outpatients (78 UC; 18 CD) for the presence of periodontal or oral mucosal disease. The results are shown in the Table. No significant difference was noted between UC and CD patients, even if stratified for sex, age, and clinical parameters. The prevalence of each disorder was similar to that seen in our enteral unit. It is difficult to compare these data with the general population, however, because of the influence of socioeconomic factors and life habits.


**No increased prevalence of malignancy in relatives of IBD patients**

G RIEGEL, R MANZIONE, F MORACE, M TARTAGLIONE, A ARIMOLI, R CARRATU (Cattedra di Gastroenterologia, 2 Universita di Napoli, Italy) The prevalence of malignancy in IBD is generally unknown in the general population. The colon in IBD patients, however, is a hallmark of inflammatory bowel disease (IBD) and colon cancer is unclear. Although colon cancer is commoner in longstanding extensive ulcerative colitis (UC) and Crohn’s disease (CD), some investigators have suggested that the proportion of family cancer patients with extensive colitis has a genetic predisposition to colon cancer and that long-standing inflammation is not itself of primary importance in the promotion of colon cancer.

We report a prospective investigation of the prevalence of malignancy in the relatives of 180 IBD patients (133 UC; 47 CD) and 120 orthopaedic patients (ORT) as controls.

The prevalence of colon cancer (5), other gastrointestinal cancer (B), and non-gastrointestinal cancer (C) has been evaluated in the first degree relatives.

In this initial study no significant difference was seen in the prevalence or spectrum of malignancy in first degree relatives of UC, CD, or ORT patients. This work was partly supported by a grant from MURST.

**Inducible nitric oxide synthase (iNOS) activity in ulcerative colitis: suppression by the anti-inflammatory cytokine IL-13**

G KOLIOS, D A F ROBERTSON, N ROONEY, R W WESTWICK (University of Bath, Department of Postgraduate Medicine and Pharmacology) Nitric oxide is an important inflammatory mediator in ulcerative colitis. We have studied the expression of its major synthetic enzyme iNOS in tissue sections using immunohistochemistry and pro-inflammatory (IL-1α, TNFα, IFNγ) and anti-inflammatory (IL-13, IL-10) cytokines that may control its activity in cell culture (HT-29 human colonic epithelial cell line).

Heavy staining for iNOS was identified in colonic epithelial cells in active ulcerative colitis (n=12) in the superficial portion of crypts and surface mucosa. No staining was seen in quiescent colitis (n=3) or controls (n=14).

In cell culture, nitrite was detected in supernatants using a fluorometric assay, iNOS mRNA by northern blot, and iNOS by western blot analysis and stained using the same antibody as in immunohistochemistry. Low basal nitrite production was considerably increased (fourfold) by pro-inflammatory cytokines IL-1α and IFNγ and up regulated (10-fold) at the post-transcriptional level by TNFα. IL-13 suppressed IL-1α/IFN iNOS activity and expression, inhibited up regulation by TNFα, and blocked increase iNOS in a synergistic manner. IL-10 had no apparent effect in this system.

Nitric oxide production and iNOS expression are closely linked to inflammation in ulcerative colitis and are susceptible to manipulation by cytokines.

**Neutrophil elastase is a secretagogue for goblet differentiated human colon cancer cells (HT29MTX)**

J D MILTON, L G YU, J DEMOCRATIS, J M RHODES (Department of Medicine, University of Liverpool, Liverpool) Goblet cell mucin depletions are a hallmark of ulcerative colitis. Elastase is a major form of the enzyme that occurs in other forms of infective and experimental colitis. The mechanism for mucous secretion and goblet cell depletion in inflamed tissue is unclear. In a previous study we demonstrated goblet cell extrusion from goblet cells (Glin Sci 86: 33) suggesting a possible mechanism for the apparent selective loss of goblet cells versus non-goblet cells that is seen in inflammatory colitis (Kaufman and Wright, J Pathol 159: 75). Further experiments have now been performed to assess the role of elastase, a known secretagogue in respiratory tissue, as a secretagogue in colonic mucosa.

The methotrexate-conditioned HT29 human colon cancer cells HT29RevMTX (kindly supplied by Dr A Zeweibam), which form well differentiated goblet cells in culture were grown on membrane inserts of a double chamber 24 well culture plate and after 7 days of confluence 1 µg 3H-glucosamine was added per well for 24 hours, after which the cells were washed to remove unincorporated radioactivity. The varying amounts of supernatant from a culture of 40×10⁶ human neutrophils incubated for 2 hours in 2 ml DEMEM+10%FCS with 1×10⁶M PMA (phorbol ester) were added to the lower wells. Four hours later the culture medium from the upper wells was removed and the secreted radioactivity counted. A dose related increase in secretion (70%) was demonstrated, which was completely inhibited by a specific inhibitor of human elastase (ICI 205, 355, 10-4M, kindly supplied by Zeneca PLC).

These data suggest that neutrophil elastase may be an important mucus secretagogue in colonic tissue.

**Understanding the mechanism of dietary therapy in Crohn’s disease – meta-analysis of polymeric enteral feeding studies and pilot study of maintenance with low fibre/low fat diet**

RICHARD C EVANS, JONATHAN M RHODES (Department of Medicine, University of Liverpool, Liverpool) There is good evidence...
that enteral feeding with formula defined liquid feeds can be as effective as corticosteroids in suppressing activity of Crohn's disease, however, it is unclear whether this is simply due to a nutritional effect. Enthusiastic uptake of this form of treatment has been hampered by high relapse rates on return to normal diet.

The aim of this study was (1) to perform a meta-analysis of published trials of polymeric feeds to assess the suggestion that response may be related to fat content, (2) to assess in a pilot study the potential use of a low fat/low fibre diet in maintenance of remission.

Nine published studies of polymeric diets contained sufficient information for analysis. Remission rates varied from 55–84% and were found to bear a strong negative correlation with %energy given as long chain triglycerides (range 1–28%, r²=0.652, p=0.009). In view of this implication that consumption of long chain fat may be harmful in Crohn's disease we have assessed the effect of low fat/low fibre and normal fat/low fibre diets in the drug free maintenance of remission in 6 patients with active ileal (2) or ileocolonic (4) disease. In each of these patients remission was induced with liquid enteral feeding (Trisorban) without corticosteroid therapy, in one with corticosteroids, and in one by low fat/low fibre diet. In the four patients who achieved maintained remission with a low fat/low fibre diet, clinical and biochemical remission is still being maintained 12, 12, 18, and 36 months later whereas two patients who took a low fibre normal fat diet had a poor response and required alternative treatment with corticosteroids or resection.

These preliminary data support the hypothesis that patients with ileal or ileocolonic Crohn's disease may not tolerate dietary long chain fat. A controlled trial is now needed to assess this formally.

Degree of sulphation and molecular weight are important for dextran sulphate and carrageenan to induce colitis in mice

L-G AXELSSON, E LANDSTRÖM, C LUNDBERG, A-C BYLUND-FELLENIUS (Department of Pharmacology, Pharmacia Pharmaceuticals AB, Uppsala; Department of Zoophysiology, Uppsala University, Uppsala, and Department of Food Science, Sveriges Kemiska och Bioskiftande Tekniska, Uppsala, Sweden) Oral dextran sulphate sodium (DSS) of 40 kDa Mw and degraded carrageenan (CAR) induce colitis in mice. To investigate the most immunologically molecular structure, DSS was synthesised with varying Mw and constant sulphation (Mw 3-7; 10; 25-6; 177; 500 kDa, 16 %S), or with constant Mw and varying sulphation (Mw 31 kDa; 3.3; 9.5; 12, 16-1 %S). CAR was degraded and sulphated (Mw ~40 kDa; 10-5, 16-6, 20-5 %S). Disease activity was measured as survival, diarrhoea, spleen enlargement, colon shortening, and oedema.

When dosage was calculated on a molar basis the effective dosage was related to the increase in molecular weight. There was a significant reduction in colitis below 16 %S. Similarly, CAR with 10-5 %S was without an immunologically effect. However, when CAR was additionally sulphated, it induced an inflammation evident in all of the clinical parameters.

In conclusion, a high degree of sulphation is crucial for DSS and CAR to be able to induce colitis in mice. However, the immunologically activity is augmented along with increasing molecular weight. In addition, to accurately compare the effects of different molecular weights, the dosage must be calculated on a molar basis.

Dextran sulphate sodium (DSS) induces experimental colitis in CD4+ cell depleted, atyhmic and NK cell depleted SCID mice

L-G AXELSSON, E LANDSTRÖM, A GRÖNBERG, A-C BYLUND-FELLENIUS (Department of Pharmacology, Pharmacia Pharmaceuticals AB, Uppsala; Department of Zoophysiology, Uppsala University, and Department of Food Science, Sveriges Kemiska och Bioskiftande Tekniska, Uppsala, Sweden) Oral DSS induces in mice a colitis which is similar to human ulcerative colitis. To investigate if immunological mechanisms were involved in DSS induced colitis, mice with different degrees of immunodeficiencies were used.

Colitis was induced in: (a) thymectomised Balb/c mice depleted of CD4+ T cells using anti-CD4 antibodies, (b) in athymic CD4+ mice lacking thymus derived T cells, (c) in T and B cell deficient SCID mice, and (d) in SCID mice depleted of NK cells using antibodies. Colitis was measured as diarrhoea, colon shortening, and histopathological damage (from TNb 0.698 to 0.313). A combination of systems (a) and (b) completely fragmented the mucin structure with a 93% drop in viscosity from TNb 0.698 to 0.048.

Dextran sulphate, 20 mg ml⁻¹, completely inhibited the drop in viscosity of colonic mucin induced by either systems (a) or (b). In the presence of both generating systems together, Dextran sulphate inhibited the fragmentation of mucin with only a 51% drop in viscosity over 24 hours.

These results show that: (a) free radicals can fragment the component mucins, leading to mucolysis of the barrier; (b) oxygen derived free radicals from infiltrating white cells will contribute to the immunologically mucous barrier in IBD; and (c) anti-diarrhoeal clays, for example, Diosmectite, will act as an antagonist of free radical induced mucolysis.

Increased faecal mucolytic activity and weakened colonic mucous gel barrier in ulcerative colitis

B J RANKIN, H J SAMSON, J P PEARSON, A ALLEN (Department of Zoophysiology, Uppsala University, Uppsala, Sweden) Faecal proteinase activity was significantly higher 4-6 (0.97) mmol N-terminals formed min⁻¹ (U) g⁻¹ dry weight faeces in patients with UC (n=97) than non-symptomatic controls 2-95 (0.60) U g⁻¹ dry weight faeces (n=77) p<0.05.

Increased faecal proteinase activity would explain the reduction in polymeric mucin content in the mucus gel leading to the observed defective mucous barrier in UC.

Mucolysis of the colonic mucus barrier by oxygen derived free radicals: implications for ulcerative colitis

J P PEARSON, D AYRE, M T DROY-LEFAIX*, A ALLEN (Department of Physiological Sciences, University of Newcastle upon Tyne, UK and *Insep Institute, Paris, France) A weaker colonic mucus barrier is associated with ulcerative colitis. Here we investigate the mucolytic activity of free radicals liberated by the white cell infiltrate associated with UC.

Mucolysis of colonic mucin 3-8 mg ml⁻¹ in PBS pH 7.4 was assessed by the drop in solution specific viscosity at 37°C over 24 hours. Free radicals were generated by two methods: (a) 0.5 mM FeSO₄, 0.5 mM ascorbic acid, and 0.5 mM EDTA; (b) 50 mM H₂O₂ and 0.05 mM FeSO₄. In the presence of either system, (a) or (b), a substantial drop of 55% in mucin viscosity (from TNb 0.698 to 0.313). A combination of systems (a) and (b) completely fragmented the mucin structure with a 93% drop in viscosity from TNb 0.698 to 0.048.

Diosmectite, 20 mg ml⁻¹, completely inhibited the drop in viscosity of colonic mucin induced by either systems (a) or (b). In the presence of both generating systems together, Diosmectite inhibited the fragmentation of mucin with only a 51% drop in viscosity over 24 hours.

These results show that: (a) free radicals can fragment the component mucins, leading to mucolysis of the barrier; (b) oxygen derived free radicals from infiltrating white cells will contribute to the immunologically mucous barrier in IBD; and (c) anti-diarrhoeal clays, for example, Diosmectite, will act as an antagonist of free radical induced mucolysis.

Bone metabolism in inflammatory bowel disease

S ARDIZZONI, S BOLLANI, P MOLTENI, V IMBIESI, E MINOLA*, M BEVILACQUA, G BIANCHI PORRO (Division of Gastroenterology and Endocrinology, "L Sacco" Hospital and *Pathological Anatomy Unit, "Niguarda" Hospital, Milan, Italy) The aim of this cross sectional study was to assess bone metabolism in 84 consecutive patients suffering from inflammatory bowel disease (IBD) (44 M, 40 F, mean age 36, range 10-74), 46 of whom had Crohn’s disease (CD) and 36 ulcerative colitis (UC). Bone metabolism was evaluated in all patients by measuring the serum concentrations of calcium (Ca), phosphate (P), parathyroid hormone (PTH), 25-hydroxyvitamin D (25(OH)D), 1,25-dihydroxyvitamin D3 (1,25(OH)D), osteocalcin (OC), carboxyterminal collagen telopeptides (ICTP), lumbar (CL), and femoral (CF) bone mineral density (BMD) by dual energy x ray absorptiometry. The scores were expressed in relation to reference values obtained in healthy young adults. A bone histomorphometry of the iliac crest bone was performed in 13 CD patients and in 6 UC patients. The serum levels of Ca, P, 25(OH)D, and 1,25(OH)D were in the
Inhibition of cytokine production and its genes, neutrophil inflammation, and tissue damage factors in DSS-colitis by human IL-1 RA

S KISHIMOTO, K HAYASHI (Institute of Health Sciences, Hiroshima University School of Medicine, Hiroshima 734, Japan) Interleukin-1 receptor antagonist (IL-1RA) is considered to suppress inflammation and the resulting mucosal damage in patients with ulcerative colitis. Colonic mucosal ulcerative colitis was induced in male Wistar rats (200 g) by oral administration of 3% DSS (MW 60000) ad libitum for 10 days. Macrophagic and microscopic injuries were evaluated, respectively, as expressed scores. Thiobarbituric acid reactive substance (TBARS), myeloperoxidase (MPO) activity, and total glutathion (GSH+GSSG) levels in the colon were determined. Colonic IL-1α, TNF-α, and GRO/CINC-1 (rat IL-8-like substance) and these mRNAs were also determined. Results: macroscopic and microscopic damages, increases in colonic MPO activity, IL-1α and GRO/CINC-1 levels were significantly suppressed by IL-1RA. Expression of both IL-1 mRNA and GRO/CINC-1 mRNA was not remarkably changed (see Table).

IL-1RA can inhibit neutrophil inflammation and tissue damage, which predisposed to colitis in the rat. This may be attributable to reduction in proinflammatory and chemotactic cytokines such as IL-1α and GRO/CINC-1 and to inhibition of free radicals.

Data represent mean (SEM), n = 6; *p<0.01 vs control, †p<0.05 vs control + DSS by Fisher’s LSD.

Arthritis in inflammatory bowel disease (IBD); an underestimated problem

B YAHUA, U DAVE, A KEAT* (St Mark’s and *Northwick Park Hospitals, Watford Road, Harrow) One hundred and one outpatients with IBD (55 Crohn’s; 46 ulcerative colitis) were referred for arthroscopy to quantify prevalence and associations. Spinal arthroscopy was recorded if continuing back pain began after age 30, or if radiological signs were present; peripheral arthroscopy if joint symptoms were present for >5 years or for >12 weeks, or if from radiological abnormalities. Arthropathy affected 31% of Crohn’s and 30% of UC patients. It was peripheral in 22% and 24% respectively, spinal in 22% and 6%. Compared with Crohn’s patients without arthropathy there was higher prevalence of smoking (59% v 29%), of perianal disease (70% v 45%), and more frequent colonic involvement (100% v 74%). In both Crohn’s and UC there was an excess of other extra intestinal manifestations in those with arthropathy (for example, erythema nodosum 28% v 3%; oral ulcers 82% v 47%; conjunctivitis 46% v 26%). Only 12 of 31 patients with arthropathy had received treatment (7 of 12 had been rheumatologists). Nonsteroidal were effective but had to be withdrawn because of intestinal side effects in half. Steroid injection of spine were used in half. Disabling joint symptoms are commoner in IBT than the literature suggests. Strong correlation with other extraintestinal manifestations, and with smoking in Crohn’s, indicate new therapeutic strategies.

Superior mesenteric artery Doppler flow: a valuable indicator of disease activity in Crohn’s disease

C HARE, M T HASSAN, C BARTRM, A FORBES (St Mark’s Hospital, Watford Road, Harrow) Increased blood flow in active Crohn’s disease is demonstrated, but reproducibility is uncertain. Measurements at the superior mesenteric artery (SMA), which is more easily defined, have therefore been performed. A total of 48 patients, including 24 with Crohn’s disease, undergoing routine abdominal ultrasound were studied. The SMA was localised with a 2-4 MHz probe (ATL probe, ATL Ultramark 9). Pulse wave Doppler at an angle of <60° determined: mean diastolic velocity (MDV), SMA area, and volume. Intraobserver variation was less than 10%; the mean of 3 values was used. Volume flow (normal <500 mI/min), SMA area, and MDV were significantly higher in Crohn’s than in non-inflammatory conditions or normal diagnostic scans. Volume flows and SMA areas followed bimodal distributions, but values were not sufficiently discriminatory for diagnostic purposes. Comparison with contemporaneous Crohn’s disease activity index (CDAI), serum C reactive protein (CRP), and global clinical assessment revealed a strong but imperfect correlation (r=0.70, p<0.005) for CDAI, but not for CRP. CDAI and CRP are imperfect tools (albeit less invasive than endoscopic/radio-isotope/pathological alternatives), and global clinical assessments correspond well to Doppler volume flows.

The Doppler results therefore provide additional and apparently independent information, and should prove of practical value in new scoring systems for Crohn’s disease.

Differential ultrastructural changes in cultured HRT-18 cells after treatment with mesalazine and dicolfenac

R DAY, P DASZAR, A FORBES (Department of Life Sciences, Kingston University and *St Mark’s Hospital, Watford Road, Harrow) Non-steroids (NSAIs) cause increased permeability and microvascular endothelial (EM). HRT-18 cells were incubated with mesalazine (500 μM) to determine if concentrations approximating to pharmacological use. The cells were then immunostained for ZO-1, a junctional protein in the terminal domain of the ZO, or subjected to routine processing for EM. All studies were performed in duplicate.

Drug treatment had no major effect on distribution of ZO-1, which remained at lateral cell margins (cell-cell contact areas), but the intensity of staining was reduced in all treated cells. EM revealed dose dependent injury: cells were packed with ribosomes, had increased vacuolisation, and clumping of nuclear chromatin. Dicolfenac also caused loss of microvilli and adhesion ‘blebbing’ effects, in contrast with chemically similar 5-aminosalicylates, which are of therapeutic value in IBD; damage to intercellular junctions is implicated. Their effects on integrity of the terminal domain of ZO-1 and a restricted cell line cell has been examined by immunofluorescence and electron microscopy (EM).

Discussion of ZO-1, a junctional protein in the terminal domain of the ZO, or subjected to routine processing for EM.
and 35 (13) ng/ml did not differ from normal (41 (14) ng/ml). However, in both active UC and CD (98 (22) and 89 (16) ng/ml) there was a pronounced increase in sE selectin. sE selectin levels correlated well with disease activity, however, the correlation with inflammatory markers (CRP/ESR) was less significant.

sE selectin levels are similarly raised both in UC and CD. sE selectin is probably shed from activated endothelium and reflects the processes attracting proinflammatory cells from the circulation into the mucosa.

Nicotine inhibition of apoptosis may play a part in the pathogenesis of Crohn's disease

M A BHATTI, H J F HODGSON (Department of Medicine, Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London) Mononuclear cells play an important part in the pathogenesis of inflammatory bowel disease (IBD) due to ongoing production of proinflammatory cytokines. If their life is prolonged by a relative lack of apoptosis changes in morphology - that is, macrophage transformation - sets up an active chronic inflammatory process. Crohn's disease (CD) is characterised by non-caseating granulomatous disorder and is more common among smokers. Therefore, we tested the effect of nicotine on apoptosis induced by variety of stimuli in peripheral blood mononuclear cells (PBMC). DNA fragmentation was measured by release of [3H]thymidine labelled DNA fragments after 24 hour incubation. Quantitative DNA fragmentation assay was confirmed by agarose gel electrophoresis. The inter and intra-assay coefficient of variation was less than 10%. The results demonstrate that the nicotine inhibits apoptosis induced by methotrexate, ultraviolet light, and TNF. The nicotinic receptor antagonists, pentolinium and hexamethonium, as well as the muscarinic antagonist, atropine did not reverse nicotine inhibition of DNA fragmentation. Our data suggest that nicotine inhibition of apoptosis does not involve the conventional nicotinic cholinergic receptors.

We have shown that the nicotine prolongs the life of PBMC by blocking apoptosis, which may be an important factor in setting up a chronic inflammatory response characteristic of CD. Inhibition of apoptosis by nicotine may also be responsible for determining the type of IBD. Further studies are required to establish whether this effect is due to an early signalling event or a direct effect on putative endonuclease responsible for DNA fragmentation.

An audit of the Aberdeen strategy in the surgical management of Crohn's disease in Grampian, north east Scotland

T J O'KELLY, D BRUCE, R A KEENAN (Ward 50, Aberdeen Royal Infirmary, Forresathill, Aberdeen) The surgical management of Crohn's disease in our unit is based upon operative strategy, which includes the following: (a) early, conservative, resectional surgery; (b) single layer interrupted appositional anastomosis; (c) wound protection and antibiotic lavage; and (d) avoidance of 'protective' stomas and mucous fistulas. During a 22 year period 445 patients have been treated in this way and data pertaining to them were collected prospectively. Some 625 operations were performed comprising 849 procedures, 581 of which were resections. The main indications for surgery were intestinal obstruction (29%), diarrhoea (29%), and chronic ill health (29%), with toxic colitis accounting for only 6% of cases. The timing of surgery was: elective/scheduled 72%, urgent 19%, and emergency 9%. Of the 581 resections, 87 involved the small bowel only, 297 both the small and large bowel, and 197 involved only the large bowel. The complications from these procedures were: delayed primary healing of a perineal wound 23%, wound infection 5%, fistula 2%, and anastomotic leak 2%. The postoperative mortality was: elective/scheduled 1%, urgent 3%, and emergency 5%. We believe these results support our management strategy and as a consequence we commend it to others.

Minimal access colostomy formation under vision

R S KIFF, J O'LEARY, HELEN BLACKWELL, M J HERSHAM (MASTER Unit, Royal Liverpool University Hospital, Prescot Street, Liverpool) Traditionally laparotomy is used to create a colostomy, but this is a major procedure particularly in frail unfit patients. However, the alternative blind trephine technique can be difficult in the obese or when the sigmoid colon is partially fixed. Laparoscopically assisted stoma formation avoids these drawbacks.

Laparoscopy is performed with a 12 mm umbilical port and two 10 mm ports (stoma site and suprapubic). The sigmoid colon is identified and mobilised by sharp dissection. A trephine is made to deliver the bowel.

Orientation is reassessed by sigmoidoscopy and the stoma fashioned. Check laparoscopy excludes bleeding and confirms correct bowel orientation.

Thirty patients, mean age 64 years have had a laparoscopic stoma fashioned for complex fistula (7), cancer (6 patients), intractable constipation (9), and incontinence (8). Mean operating time was 46 minutes (range 25-95), postoperative stay 6-7 days (range 3-20 days). Twenty seven patients were eating after 2 days and 2 further patients after 4 days. One patient with advanced malignancy died from pneumonia, and one patient developed a parastomal hernia.

We have found that in our experience laparoscopic stoma formation is safe and easy to perform in most patients. This operation is performed under vision and avoids a laparotomy wound.

Hileoectomy for Crohn's disease - a minimal access approach

R S KIFF, A GHANAEI, J O'LEARY, M J HERSHAM (MASTER Unit, Royal Liverpool University Hospital, Prescot Street, Liverpool) Limited ileocectomy is often necessary for Crohn's disease not controlled by medical therapy. A minimal access approach offers lower morbidity.

Laparoscopy is performed using three midline 12 mm ports (epigastric, umbilical, and suprapubic). The disease area is assessed and compared with radiological observations. Sufficient distal ileum, and right colon is mobilised by scissor dissection. Diseased bowel is delivered through a small right side incision. Resection and anastomosis is performed extraperitoneally.

Twenty patients (12 female, 8 male) mean age 33-6 years (range 22-59) underwent ileocectomy for Crohn's disease after medical treatment on a gastroenterology unit. The indication was ileal stricture (11 patients), pain (5 patients), a mass (2 patients), recurrent disease with a stricture (1 patient), and stricture with ileosigmoid fistula (1 patient). Mean overall operating time was 105 minutes (60-175 minutes). Mean postoperative stay was 5 days (3-9 days). One patient developed an abscess requiring percutaneous drainage. One patient has developed recurrent Crohn's after 20 months.

We found that laparoscopically assisted right ileocectomy is associated with low morbidity, a small wound, quick patient recovery, and short length of stay. It is relatively simple surgery with advantages to patients.

International Inflammatory Bowel Disease

A640

Gut: first published as 10.1136/gut.38.4.A635 on 1 April 1986. Downloaded from http://gut.bmj.com/ on September 16, 2023 by guest. Protected by copyright.