FAecal Lactoferrin in Inflammatory Bowel Disease
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Serum levels of lactoferrin, released by inflammatory cells during phagocytosis, have been shown to correlate with disease activity in inflammatory bowel disease. Since faecal lactoferrin might be more reflective of tissue activity, this was measured by ELISA and compared with α-1 antitrypsin (by immunoturbidimetric assay) as a marker of protein loss.

Fresher voided faecal samples from normal controls (n=17) patients with Crohn's disease (n=12) and ulcerative colitis (n=24) were homogenized, centrifuged and a bacterial free supernatant obtained by passage through a series of filters culminating in a 0.2μm filter. Activity of inflammatory bowel disease was measured by clinical criteria and simple activity index for ulcerative colitis and Crohn's disease respectively.

Faecal lactoferrin was markedly raised in both ulcerative colitis (median 680, range 307-1021 ng/mg) and Crohn's disease (478, 169-3048 ng/mg) compared to controls (2.5, 0.6-6.7 ng/mg) (P<0.0001), when measured per mg protein content. The lactoferrin/α-1 antitrypsin ratio calculated as a measure of inflammation was similarly raised in both ulcerative colitis (median 0.0046, range 0.0117-0.1037 g/g) and Crohn's disease (0.0585, 0.0107-0.165) compared to controls (0.0022, 0.0005-0.0008) (P<0.0001). There was significant correlation with disease activity in both ulcerative colitis (P<0.01) and Crohn's disease (P<0.01). There was no relationship between faecal lactoferrin levels and α-1 antitrypsin levels.

The faecal lactoferrin/α-1 antitrypsin ratio is easily measured and should be a useful indicator of inflammatory bowel disease activity.

THE EFFECT OF AMINOSALICYLATES AND POTENTIAL NEW DRUGS ON MUCOCAL REACTIVE OXYGEN METABOLITE PRODUCTION IN ULCERATIVE COLITIS.
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The therapeutic efficacy of the aminosalicylates in ulcerative colitis is thought to be due, at least in part, to their ability to scavenge reactive oxygen metabolites (ROM) and inhibit myeloperoxidase (MPO). We have previously used luminol-amplified chemiluminescence to show increased rectal mucosal ROM production in vitro in ulcerative colitis (Gut 1991; 32: A589). We have now used this system to assess the antioxidant effects of 5-ASA (20mM), and the potential of other compounds to inhibit the ROM production in ulcerative colitis, and compared their effects in this system to that in cell-free systems using xanthine (X) (50μM) / xanthine oxidase (XO) (0.017U/mL) with or without myeloperoxidase (0.05U).

RESULTS: These are expressed as the mean % change (SD) in chemiluminescence when compared to buffer. Significance assessed using analysis of variance and Fisher's LSD.

$$\begin{array}{c|c|c|c}
\text{Rectal mucosa} & \text{5-ASA} & \text{5-ASA} & \text{5-ASA} \\
\text{(20mM)} & \text{5-ASA} & \text{5-ASA} & \text{5-ASA} \\
\text{(2mM)} & \text{(2mM)} & \text{(2mM)} & \text{(2mM)} \\
\text{XOX} & \text{XOX} & \text{XOX} & \text{XOX} \\
\text{(n=3)} & \text{(n=3)} & \text{(n=3)} & \text{(n=3)} \\
5-ASA \text{ (20mM)} & -93 \text{ (6)} & -107 \text{ (4)} & -114 \text{ (4)} & -123 \text{ (3)} \\
5-ASA \text{ (2mM)} & -59 \text{ (22)} & -102 \text{ (5)} & -96 \text{ (2)} & -111 \text{ (3)} \\
\text{Balsalazide (20mM)} & -100 \text{ (56)} & -128 \text{ (5)} & -111 \text{ (3)} & -118 \text{ (4)} \\
\text{Taurine (20mM)} & -32 \text{ (60)} & -67 \text{ (5)} & -62 \text{ (3)} & -111 \text{ (3)} \\
\text{Ascorbate (20mM)} & -52 \text{ (21)} & -117 \text{ (7)} & -117 \text{ (7)} & -122 \text{ (3)} \\
\text{N-acetyl cysteine (10mM)} & +44 \text{ (45)} & -104 \text{ (4)} & -102 \text{ (1)} & -102 \text{ (1)} \\
\end{array}$$

*p<0.05 vs. buffer control, tp<0.05 vs. 5-aminosalicylic acid (20mM for rectal mucosa; 0.2mM for cell free systems), tp<0.05 vs. effect of dose on rectal mucosa.

CONCLUSIONS: 1) Measurement of the effect of drugs on the chemiluminescence produced by rectal biopsies is a useful way of screening potential antioxidant and antimicrobial agents in ulcerative colitis.
2) Endogenous agents such as taurine, ascorbate and N-acetyl cysteine appear to be less potent in our system than might be anticipated from their antioxidant activity in vitro and less effective than the aminosalicylates. 3) The new aminosalicylate, balsalazide, is as effective as 5-aminosalicylic acid in this system, suggesting that antioxidant activity may be important in its mechanism of action.

NEITHER 99m-TECHNETIUM-HMPAO WHITE CELLS NOR 99m-TECHNETIUM NANOCOLLOID ARE SUFFICIENTLY SENSITIVE TO RECOMMEND THEIR USE IN THE ASSESSMENT OF COLONIC INFLAMMATORY BOWEL DISEASE
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99m-Technetium has considerable theoretical advantages over 111-Iodium-labeled albumin labelling in that it is more readily available, less time consuming and offers a more readily acquired scan energy for modern gamma cameras.

Twenty patients with biopsy proven Ulcerative Colitis were examined to detect the extent and activity of mucosal inflammation by nanocolloid and white cell scanning. Subjects were divided into two groups, the first scanned with 148 MBq 99m-Tc nanocolloid on day one followed by 370 MBq 99m-Tc white cells 26 hours later. Group two subjects were scanned in reverse order. Both groups were colonoscoped immediately after the second scan when a visual assessment of extent and degree of inflammation was made and each of seven colonic areas was biopsied for histology.

Scan results were correlated with the visual assessment at colonoscopy and the histological degree of chronic inflammatory cell infiltrate (the absolute indication of inflammation). Nanocolloid scanning was shown to be 35.7% sensitive compared with visual inflammation and 31.8% sensitive compared with histology with an average specificity of 94.2%. Sensitivity of white cell scanning was 58.5% compared with visual assessment and 49.4% compared with histology with an average specificity of 96.9%. Colonoscopic assessment was 73.8% sensitive when all degrees of inflammation were considered, rising to 92.6% when the most mild degree of inflammation was excluded. No scanning artefacts were seen due to bowel preparation.

Neither method of angiographic detection of mucosal inflammation is sufficiently sensitive to recommend their routine use in the assessment of colonic inflammatory bowel disease.

MONONUCLEAR CELL PROCOAGULANT ACTIVITY IN INFLAMMATORY BOWEL DISEASE.
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Thrombogenesis is activated in patients with Crohn's disease (CD). AIM: To examine mononuclear cell procoagulant activity (MPCA) in patients with CD and to study the MPCA response to autologous serum (AS) on the induction and expression of MPCA. METHODS: Spontaneous MPCA was measured in peripheral blood mononuclear cells (PBMC) inhibited by patients with active CD (Harrow-Bradshaw score >4; n=46), inactive CD (n=56), patients with ulcerative colitis (n=19) and normal controls (n=50). In addition, MNC from patients with CD and controls were incubated with concentrations of AS and bacterial lipopolysaccharide (LPS; 1ug/mL) for 4h at 37°C. The MPCA was measured in a modified 3-stage clotting assay and expressed as μM/2x10⁶ MNC. RESULTS (mean±SEM): MNC from patients with active CD expressed significantly more spontaneous MPCA (14±3.3 μM) than normals (0.6±0.3 μM), inactive CD (1.4±0.4 μM) or ulcerative colitis (2.2±2 μM), p<0.001. The results of MPCA after 4h incubation were:

<table>
<thead>
<tr>
<th>MNC ALONE</th>
<th>MNC+LPS</th>
<th>MNC+AS</th>
<th>MNC+LPS+AS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crohn's</td>
<td>196±45</td>
<td>351±206</td>
<td>504±245</td>
</tr>
<tr>
<td>Normal</td>
<td>26±8</td>
<td>79±12</td>
<td>28±15</td>
</tr>
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*p<0.05 compared with normal controls. T-test.

There was no significant difference between active and inactive Crohn's disease in the 4h MPCA response (Mann-Whitney U p>0.05). However, sera from 12 of the 45 CD patients inhibited the expression of MPCA induced by LPS alone (LPS+AS = 74±21; p<0.001). CONCLUSIONS: MNC in active CD are spontaneously procoagulant. Sera from the majority of CD patients contain MPCA stimulatory activity that is synergistic with LPS, but a minority of sera inhibit MPCA expression that may protect against thrombosis in Crohn's.
ANTIBODIES TO MYCOBACTERIUM PARATUBERCULOSIS AND NINE SPECIES OF ENVIRONMENTAL MYCOBACTERIA IN CROHN'S DISEASE AND CONTROLS

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Interest has focussed recently on the possible role of Mycobacterium paratuberculosis in the pathogenesis of Crohn's disease. Culture and serological studies have provided conflicting evidence for the involvement of this organism. We have attempted to determine whether a specific serum antibody response, to a range of mycobacterial species, occurs in Crohn's disease. Sera from patients with active (n=24) or inactive Crohn's disease (n=29), ulcerative colitis (n=15) and controls (n=30) were assayed in enzyme-linked immunosorbent assays for total immunoglobulin levels to eight filtered sonicate mycobacterial preparations and purified protein derivatives of M. paratuberculosis and the bovine tubercle bacillus. IgG, IgM and IgA levels to M. paratuberculosis were also determined.

There was strong evidence of contact with environmental mycobacteria in all patient and control populations but with no statistical differences between total immunoglobulin levels. Responses were greatest to preparations of M. avium, M. tuberculosis and M. kansasii. There were no differences in IgG levels to M. paratuberculosis in Crohn's disease (mean=0.23, s.d.=0.16) and controls (mean=0.21, s.d.=0.16; p=0.05) or controls (mean=0.20, s.d.=0.16). Despite a subset of patients with active Crohn's disease (6/24), possessing positive IgG levels to M. paratuberculosis (exceeding the mean±2 s.d. of the control population). IgA and IgM levels were similar in all groups.

This study indicates that virtually all individuals possess antibodies to environmental mycobacteria. Patterns of antibody levels in patient and control populations are similar to those observed with other organisms: These results do not provide convincing serological evidence of a role for M. paratuberculosis in the pathogenesis of Crohn's disease. It remains to be determined whether M. paratuberculosis specific antigen preparations would differentiate between patient and control groups.

IN VITRO C-REACTIVE PROTEIN SYNTHESIS IN INFLAMMATORY BOWEL DISEASE
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C-reactive protein (CRP) is an acute phase reactant. In response to inflammation cytokines directly induce CRP synthesis by the liver. We assessed the ability of monocyte conditioned medium with and without lipopolysaccharide (LPS)-stimulation to release CRP from a human hepatoma cell line, Alexander cells, and also measured serum CRP in 22 patients with Crohn's disease(CD), 22 patients with ulcerative colitis(UC) and 11 healthy controls.

Serum CRP was significantly higher in patients with CD (29.7 ± 7.9 mg/dl) compared to UC (6.8 ± 1.6 mg/dl; p<0.05) and normal controls (6.0 ± 0.5 mg/dl; p<0.001). Both unstimulated and LPS-stimulated monocyte conditioned medium from patients with CD (1.26 ± 0.24 ng/ml and 2.49 ± 0.07 ng/ml respectively) caused greater CRP release from Alexander cells than conditioned medium from UC (0.68 ± 0.11 ng/ml; p<0.05 and 2.04 ± 0.21 ng/ml; p<0.05 respectively) and normal controls (0.61 ± 0.09 ng/ml; p<0.05 and 1.30 ± 0.12 ng/ml; p<0.05 respectively), although the difference was significantly more for unstimulated CM than LPS-stimulated CM.

These findings are accord with other reports that serum concentrations of CRP is significantly more elevated in patients with CD than in UC. The greater expression of CRP in Crohn's disease corresponds to greater production of CRP by hepatocytes in response to CD monocyte conditioned medium which may reflect qualitative differences in the systemically active cytokines produced in Crohn's disease compared to ulcerative colitis.

MUCOSAL INTERLEUKIN-6 PRODUCTION IN INFLAMMATORY BOWEL DISEASE
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The cytokine interleukin-6 (IL-6) is an important mediator of the inflammatory response. Raised circulating levels have been found in the plasma of the majority of patients with active Crohn's disease and in some patients with active ulcerative colitis.

To investigate possible mucosal origin of circulating IL-6, mucosal biopsies taken at colonoscopy or sigmoidoscopy from patients with active (n=10) and inactive (n=7) ulcerative colitis, active Crohn's disease (n=4) and controls (n=11) were cultured in vitro for 24 hours. Secreted IL-6 was determined by ELISA and results expressed as IL-6 production per mg tissue per 24 hours. Biopsies taken from an immediately adjacent area were examined histologically. The acute and chronic inflammatory cell infiltrates in ulcerative colitis biopsies were graded on a subjective semi-quantitative scale by a single observer.

IL-6 production from patients with active ulcerative colitis was significantly greater (median = 1,572 pg IL-6/mg tissue, range 466-7,193) than patients with inactive ulcerative colitis (median = 206, range 111-846, p<0.001), active Crohn's disease (median = 360.3, p<0.001) and controls (median = 63, range 15-558, p<0.001). In active ulcerative colitis, there was no correlation between the endoscopic appearance of the mucosa (Baron score) and IL-6 production. IL-6 secretion by ulcerative colitis biopsies did correlate with both acute (R = 0.77, p<0.001) and chronic inflammatory cell infiltrate (R = 0.83, p<0.001).

These results suggest that IL-6 is secreted locally by the inflamed colonic mucosa in ulcerative colitis, despite the lack of elevated plasma levels in the majority of patients.

CROHN'S DISEASE: GRANULOMATOUS LYMPHANGITIS RATHER THAN VASCULITIS
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Recent studies have suggested that Crohn's disease is a primary vasculitic process. To determine precisely which types of vessels are involved we have investigated the relationship of granulomas with blood and lymphatic vessels and have sought fibrinoid necrosis and inflammatory cell infiltration of the vessel wall.

Methods: Resected tissue from active Crohn's disease from 10 patients (3F, aged 18-67) was studied (small bowel in 6 and large bowel in 4 patients). Paraffin sections were stained with H&E, Martius scarlet blue (for fibrin) and by immunoperoxidase for vascular endothelium (QBend10 and UEA lectin) and macrophages (KP1).

Granulomas observed on H&E were examined in parallel sections to determine the nature of involved vessels and the presence of vasculitis.

Results: 149 granulomas from all 10 patients were examined. Most were shown by UEA to be closely associated with small blood vessels lined by endothelium, whose immunocytochemical and morphological characteristics suggested that they were lymphatic rather than blood vessels. Many granulomas lay within these lymphatics. Impingement of granulomas on neighbouring vessel walls involved vasa vasorum, not arteries and was uncommon. In none of the vessel walls was there fibrinoid necrosis or other evidence of vasculitis.

Conclusions: The pathological changes seen in Crohn's disease do not meet the traditional criteria of vasculitis. On the contrary, the evidence is more in favour of a granulomatous lymphangitis.