MITOCHONDRIAL FUNCTION OF COLONIC EPITHELIAL CELLS IN INFLAMMATORY BOWEL DISEASE

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In active inflammatory bowel disease there is epithelial cell damage, and consequently increased permeability to luminal contents. Colonocyte metabolism relies on mitochondrial oxidative phosphorylation with butyrate as carbon-source; butyrate usage is impaired in active ulcerative colitis (UC). To investigate whether colonocyte damage results in impaired mitochondrial biogenesis, we have studied mitochondrial function in inflammatory bowel disease.

Activity of respiratory chain enzymes cytochrome oxidase and succinate dehydrogenase was assessed on cryostat sections using histochemical hybridisation shows cytokeratin may be detected is derived from segments of histological biopsy using antibodies specific for cytokeratin in patients with segmental Crohn's disease. Respiratory chain activity was predominately in the epithelial layer, and in each case was increased in colonocytes of biopsies from active disease compared with a control biopsy from an inactive region from the same patient. In vitro activity of cytochrome oxidase in homogenised biopsies from patients with active ulcerative colitis (corrected for epithelial content by cytokeratin estimation) was also increased (0.057±0.017 mg protein, p<0.05) compared with control patients p=0.05 (0.057±0.017 mg protein, n=50). Furthermore, in situ hybridisation shows increased levels of mitochondrial chromosomal mRNA bands of colonocytes in biopsies of active ulcerative colitis (n=16) or Crohn's colitis (n=9) compared with the same patient in remission or control patients (n=18).

These results suggest that mitochondrial biogenesis and respiratory chain activity is increased in patients with active inflammatory bowel disease. This response is appropriate to the increased energy requirement of these cells, and indicates that mitochondrial function does not impair colonocyte metabolism. Increased mitochondrial biogenesis may result from increased expression of heat-shock proteins required for functional mitochondrial assembly during inflammation.

INCREASED PRODUCTION OF SUPEROXIDE BY RECTAL MUCOSA IN INFLAMMATORY BOWEL DISEASE.

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Reactive oxygen metabolites (ROMs) are thought to be important in the pathogenesis of inflammatory bowel disease (IBD). We have previously used luminol-amplified chemiluminescence (CL), which detects ROMs in the presence of myeloperoxidase, to show increased rectal mucosal production of ROMs in vitro in IBD (Gut 1991; 32: A589). We have now used lucigenin-amplified chemiluminescence to detect ROMs in conjunction with superoxide dismutase (300U) and the xanthine oxidase inhibitor, oxyuripyrin (10^-4M), to investigate the production of superoxide by the rectal mucosa in IBD.

RESULTS Lucigenin- and luminol-amplified CL, expressed, in photons/min/mg wet weight x 10^4, as median values with 95% confidence intervals or ranges where n=5, were increased in both ulcerative colitis (UC) and Crohn's disease (CD) compared with controls (Kruskal-Wallis p<0.05) and increased with macroscopic disease activity, n Lucigenin-amplified n Luminol-amplified chemiluminescence chemiluminescence

Control 24 5 (4-6) 80 1 (0.4-2)
UC (normal) 6 10 (3-6) 36 1 (0.7-5)
UC (Pancreatic) 10 37 (2-85) 61 36 (4-69)
UC (Oxycetin) 7 24 (4-29) 44 126 (65-169)
UC (3 spontaneous) 2 69 (42-96) 16 201 (94-362)

CL (normal) 3 12 (4-38) 16 1 (0.6-6)
CL (mild) 5 8 (5-66) 12 9 (3-34)
CL (severe) 9 24 (6-120) 5 106 (25-789)

Lucigenin- and luminol-amplified CL were directly related (Spearman's p=0.62, p<0.001). In UC, lucigenin-amplified CL was decreased by the addition of SOD (-63%, n=6, p<0.05) and oxyuripyrin (-26%, n=5, p<0.05).

CONCLUSIONS 1) Superoxide is produced by the rectal mucosa in IBD and can be detected using lucigenin-amplified CL. 2) That SOD inhibits CL more than oxyuripyrin suggests that most of the superoxide detected is related to leukocytes, rather than from xanthine oxidase.

These results suggest that further evaluation of the therapeutic role of SOD in IBD is worthwhile.
Endothelial cells play a crucial role in angiogenesis, representing an essential event in different chronic inflammatory processes. Therefore we investigated the state of endothelial cell activation and differentiation in chronic IBD. For the first time the monoclonal antibodies for endothelial cell differentiation and malignant transformation MS-1, 1F10 and A10-33/1 (Goedert et al., Exp.Cell.Biol.57:185,1989) were studied in the human intestine.

We used the indirect peroxidase method to analyse the phenotypic expression of six endothelial markers on frozen sections from patients with IBD (7 Crohn's disease, 6 ulcerative colitis), who had undergone colonoscopy.

We could show that in histologically "acute" inflammation endothelial cells strongly express 1F10, a marker for continuously growing cells. In contrast, we found expression for MS-1, a monoclonal antibody for discontinuously growing endothelial cells. As reported by others (Malizia et al., Gastroenterology 100:160,1991), we found a strong expression for ICAM-1 in the histologically "acutely" inflamed mucosa. With A10-33/1, an endothelial cell marker shown to be strongly expressed on melanoma cells, we found similar expression in the inflamed mucosa or under control conditions. No immunohistochemical staining was encountered in the mucosa with VCAM-1 and ELAM-1.

Thus we have shown for the first time that in histologically "acute" IBD an upregulation exists only for those endothelial cell antigens which are already expressed in the control mucosa (ICAM-1, 1F10). The missing expression for the discontinuously growing endothelial cell marker MS-1 and the uniform expression of the melanoma associated A10-33/1 led us to conclude that in the case of IBD (1) endothelial cells do not loose their differentiation potential and (2) regulation of angiogenesis is maintained.

WHAT ANTIGEN REACTS WITH ANTI NEUTROPHIL CYTOPLASMIC ANTIBODY IN INFLAMMATORY BOWEL DISEASE AND PRIMARY SCLEROSING CHOLANGITIS?

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Anti neutrophil cytoplasmic antibodies (ANCA) are present in the serum of some patients with Inflammatory bowel disease (IBD) and Primary sclerosing cholangitis (PSC). All of this study was to measure the prevalence of ANCA in this population, its relationship to disease extent and activity and to identify the cytoplasmic antigen. IgG ANCA was measured using indirect immunofluorescence on ethanol fixed leucocytes. The cytoplasmic (c) and perinuclear (p) patterns were determined. Antibodies to Cathepsin G (CG) and Myeloperoxidase (MPO) were measured by ELISA, 42 controls, 28 patients with Crohn's disease, 19 patients with Ulcerative colitis 3 patients with PSC and 26 patients with other liver diseases were studied. In Crohn's disease, 16% were PANCA positive and 35% were CG positive. PANCA was positive in 38% of UC, 33% of PSC, 15% of liver controls and 0% of control patients. ANCA was present in 17% of UC, 33% of PSC and 15% of liver controls. MPO was rarely positive in any group. ANCA positivity was unaffected by inflammatory bowel disease duration, extent or activity.

Conclusion. pANCA and cANCA positivity is commoner in UC than Crohn's disease, but is unaffected by disease duration, extent or activity. Cathepsin G but not myeloperoxidase appears to be the major antineutrophil cytoplasmic antigen in IBD.

INTERACTIONS BETWEEN INTERLEUKIN 1 AND PROPHOSPHOLIPID DERIVATIVES IN ULCEARTIVE COLITIS. W.M. Patek, D.J. Warburton

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Cytokines, in particular interleukin 1 have been implicated in the pathophysiology of ulcerative colitis (UC) however the precise effects of these cytokines remain in doubt. METHODS: Thirty patients with active UC had biopsies taken from inflamed rectum. Rectal biopsies were also taken from age and sex matched controls with irritable bowel syndrome. Biopsies were cultured for 4 hours, either (a) untreated or with (b) interleukin 1, (c) mepacrine, (d) a phospholipase A2 inhibitor (10^-4M). Culture medium was assayed for prostaglandin E2 (PGE2), leukotriene C4 (LTC4), platelet activating factor (PAF) and interleukin 1a. RESULTS: (pg/mg wet weight/hr.)

<table>
<thead>
<tr>
<th>IL1a</th>
<th>PGE2</th>
<th>LTC4</th>
<th>PAF</th>
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<tbody>
<tr>
<td>Control</td>
<td>a) Untreated</td>
<td>1.74±0.1</td>
<td>19±18</td>
</tr>
<tr>
<td></td>
<td>b) IL1a</td>
<td>38±26</td>
<td>29±27</td>
</tr>
<tr>
<td></td>
<td>c) Mepacrine</td>
<td>1.56±0.6</td>
<td>38±24</td>
</tr>
<tr>
<td>Inflamed</td>
<td>a) Untreated</td>
<td>16.4±0.4</td>
<td>7±5</td>
</tr>
<tr>
<td></td>
<td>b) IL1a</td>
<td>94±45</td>
<td>45±35</td>
</tr>
<tr>
<td></td>
<td>c) Mepacrine</td>
<td>13.4±14</td>
<td>5±3</td>
</tr>
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*Significantly different from untreated value p<0.01
**Significantly different from untreated value p<0.001

Inflamed tissue released significantly more PGE2, LTC4, and IL1a into the culture medium when compared with control tissue. Only inflamed tissue produced PAF. Interleukin 1a significantly increased the production of PGE2, LTC4 and PAF in both inflamed and control tissue. Phospholipase A2 inhibition with mepacrine virtually abolished the release of these compounds.

CONCLUSION: The effects of interleukin 1a in ulcerative colitis may be mediated by phospholipase A2 derivatives including PGE2, LTC4, and PAF.