
Both Nitric Oxide synthase and Cyclooxygenase exist in constitutive and inducible forms (iNOS and COX II respectively). The co-expression of the inducible isoforms of these enzymes was investigated in colonic specimens from patients with active ulcerative colitis (n=6) and in patients undergoing resection for colorectal carcinoma (n=4). Tissues were immediately frozen in liquid nitrogen and post-fixed in buffered 1% paraformaldehyde as required. RT-PCR using specific primers for iNOS and COX-II was performed. PCR products were sequenced and then used to generate radio-labeled single stranded DNA probes for in situ hybridisation (ISH) in order to investigate the localisation of iNOS and COX II mRNA.

ABC immunostaining techniques using mouse polyclonal iNOS, COX II and COX I antibodies were performed. Qualitative RT-PCR revealed the presence of both iNOS and COX II mRNA in all of the specimens examined. ISH showed iNOS mRNA concentrated in the epithelial layer. In ulcerative colitis, but not in normals, iNOS immunostaining was positive in the colonic epithelium, infiltrating cells of the submucosa and smooth muscle cells. COX I immunostaining was predominantly in the epithelium and was similar in ulcerative colitis and normals, but epithelial COX II immunostaining was greatest in ulcerative colitis.

These preliminary results suggest that the expression of iNOS and COX II in the colonic epithelium may be important in the pathogenesis of ulcerative colitis.

Immunology and inflammation T116–T123

The role of Nitric Oxide (NO) Cholera Toxin (CT)-induced Small Intestinal Secretion in Rat. J. Turvill, MJG Farthing. Digestive Diseases Research Centre, St. Bartholomew’s & The Royal London School of Medicine & Dentistry, London.

Introduction. The role of NO in mediating small intestinal secretion remains uncertain. Its action on epithelial permeability, vascular tone, the enteric nervous system and mast cell and neutrophil function are complex and appear at times to be paradoxical.

Aim. We sought to determine the effect of the NO precursor, L-Arg and the NO synthase inhibitor L-NNAME on CT-induced secretion.

Methods. Exp 1: Under phenobarbitone anesthesia rats were pretreated with saline, 20mg/kg or 40mg/kg L-NNAME sc or 500mg/kg L-Arg sc. 75µg CT was instilled into whole small intestine isolated between cannules. After 2h in situ perfusion was performed using plasma electrolyte solution with a non-absorbable marker (¹⁴C-PEG) to assess net water and electrolyte movement.

Exp. 25cm jejunal segments were incubated with 25µg CT. 20mmol/L-NNAME or L-Arg was added to the perfusate.

Results. Paradoxically, CT-induced secretion (med -85.5±ml/min/g [IQR -69.0 to -101.4], n=13) was reduced by both sc L-NNAME (dose-dependently) (20mg/kg: -54.5 [-39.1 to -71.1], n=8; p<0.002. 40mg/kg: -3.0 [12.0 to -23.5], n=9; p<0.001 compared to control and 20) and by 500mg/kg L-Arg (-69.9 [-73.7 to -63.7], n=9; p=0.0006). 40mg/kg L-NNAME was more effective than L-Arg in reducing secretion (-101.2 [-74.9 to -146.5], n=14) was not altered by addition of L-NNAME (-94.3 [-80.2 to -117.2], n=9) or L-Arg (-113.6 [-77.6 to -148.9], n=8) to the perfusate.

Conclusion. These apparently paradoxical findings point to a balance of influences of NO that exerts in the control of CT-induced secretion. We suggest that L-NNAME suppresses secretion by impairing mesenteric blood flow and that L-Arg acts by inhibiting both mast cell degranulation and enteric neural activation.
INCREASED INDUCIBLE NITRIC OXIDE SYNTHASE mRNA AND PROTEIN IN THE SMALL INTESTINE OF PATIENTS WITH UNTREATED COELIAC DISEASE.


Introduction: It was previously demonstrated that there is increased NADPH-diaphorase staining in the lamina propria of patients with untreated coeliac disease (CD). This histochemical technique is not specific for the inducible isoenzyme of nitric oxide synthase (iNOS). Aims: Our aim was to determine whether iNOS is the isoenzyme responsible for the increased NADPH-diaphorase staining in jejunal biopsies from patients with CD compared to controls.

Methods: Small intestinal biopsies from 18 patients with CD, 9 treated (CD/GFD) and 9 untreated (CD/ND) and 8 disease controls (DC) were studied. Cryostat sections from jejunal biopsies were incubated with a polyclonal antibody to inducible NOS and stained by immunohistochemistry (IHC). In situ hybridisation (ISH) was performed using a non-radioactive oligonucleotide probe cocktail. Sections were assessed blindly by two independent observers and the lamina propria staining counted with an eye-piece graticule per 0.1 mm².

Results: No positive cells were noted in the epithelium with either method. The results in the lamina propria are given below:

<table>
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<tr>
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<th>CD/GFD</th>
<th>CD/ND</th>
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<tbody>
<tr>
<td>mRNA</td>
<td>median 33.5</td>
<td>47</td>
</tr>
<tr>
<td>range</td>
<td>18-38</td>
<td>19-82</td>
</tr>
<tr>
<td>IHC:</td>
<td>median 9.4</td>
<td>12.5</td>
</tr>
<tr>
<td>range</td>
<td>4-11.7</td>
<td>6-20</td>
</tr>
</tbody>
</table>

There was significantly greater iNOS mRNA expression in the lamina propria of patients with CD/ND compared to those with CD/GFD and DC (p=0.002) and DC (p=0.002) but no difference between CD/GFD and DC (p=0.04). The methods correlated significantly, with Kendall’s Tau=0.478, p=0.002.

Conclusion: Using two different methods, we have demonstrated significantly raised levels of iNOS mRNA and protein in patients with untreated CD. This supports a role for nitric oxide in the pathogenesis of the observed small intestinal damage in CD.

ASSOCIATION OF ANTRAL MUCOSAL PRODUCTION OF INTERLEUKIN-8 AND REACTIVE OXYGEN RADICALS IN PATIENTS WITH HELICOBACTER PYLORI INFECTION.

Zhong, J.B., Dawodu, A., Hussain, O., Etoh, C., Gemmell, R.J., Russell, Department of Gastroenterology and Bacteriology and Pathology, Royal Infirmary, Glasgow G31 2ER

Helicobacter pylori (Hp) infection is characterised by an infiltration of neutrophils in the gastric mucosa. There is evidence that in Hp infected patients there is an increased mucosal production of interleukin-8 (IL-8). However, the relationship between IL-8 and reactive oxygen radicals (ROR) remains to be clarified.

Aims: To investigate if there is any association between antral mucosal production of IL-8 and ROR and their relationship to gastric antral inflammation.

Methods: 52 patients referred for endoscopy were recruited. Gastric antral biopsies were taken for measurement of IL-8 and chemiluminescence (CL, measuring ROR), histology and culture. IL-8 was measured by ELISA and the result expressed as pg/mg of biopsy. Luminol-enhanced CL was measured by luminometry and results expressed as light signal/minute of biopsy. Antral inflammation was assessed by a pathologist in a blinded fashion.

Results: IL-8 concentrations were significantly increased in Hp colonised antral mucosa (0.88±0.11 pg/mg (mean±SE)) than Hp negative ones (0.2±0.06 pg/mg, p<0.001). Hp colonised mucosa also displayed a higher CL response (signal/min/mg) than Hp negative ones (1.7±0.285 vs 3.12±0.9, p<0.01). There was a positive correlation between IL-8 concentration and CL response in the antrum mucosa (r=0.72). Greater neutrophil infiltration was associated with a higher IL-8 concentration (p<0.0001) and a stronger CL response (p<0.007).

Conclusion: IL-8 concentration is correlated with the production of ROR in antral gastric mucosa and is associated with an infiltration of neutrophils. IL-8 may be important in attracting and activating neutrophils to release ROR and to cause mucosal inflammation in Hp colonised patients.

REGULATION OF INTERLEUKIN-8 PRODUCTION IN GASTRIC EPITHELIAL CELLS STIMULATED BY HELICOBACTER PYLORI, INTERLEUKIN-1 AND TUMOUR NECROSIS FACTOR-α

John L.P. Bates and John Calam

Gastroenterology Unit, Royal Postgraduate Medical School, London, UK

Production of interleukin-8 (IL-8) by gastric epithelial cells is believed to be important in the initiation and perpetuation of gastric inflammation and the chronic process in patients with H. pylori (Hp) infection. Whilst understanding of the bacterial factors involved is increasing, less is known of the pathways involved in IL-8 secretion by the epithelial cells.

Therefore we have used specific stimulants and inhibitors of intracellular protein kinase pathways to determine the mechanisms involved in IL-8 production.

Methods: Human gastric epithelial carcinoma (AGS) cells were co-cultured with Hp or exposed to IL-1β or TNFα as well specific activators or inhibitors. IL-8 production was measured by ELISA.

Results: Significant stimulation of IL-8 production was seen following activation of protein kinase C (PKC) with the phorbol ester, phorbol myristate (PMA, 10nM) (18.5 fold increase over basal), or, with stimulation with 1 ng/ml IL-1β (22.5 fold) or TNFα (19 fold) of two ulcerogenic Hp strains (12.5 and 5.8 fold increases respectively) (all p<0.001). Neither activation of protein kinase A with forskolin or dibutyryl-cAMP nor inhibition with H89 had any effect on IL-8 production under any conditions. The stimulatory action of PMA was blocked by the PKC inhibitor H-7 (100 μM) by prior PKC depletion by 24 hours pre-exposure to PMA 500nM. Neither of these manoeuvres had any inhibitory action on the stimulation produced by Hp, IL-1β or TNFα. The tyrosine kinase inhibitors herbimycin A (200nM) or genistein (100 μM) significantly impaired the IL-8 production induced by Hp (88 and 99% inhibition of stimulation respectively; p<0.001). IL-1β (by 41 and 51 μM; p<0.02) and TNFα (by 62 and 69 μM; p<0.001). Directly elevating intracellular calcium with the ionophore A23187 (1 μM) produced a weak IL-8 response (IL-8 but significantly potentiated the effect of phorbol myristate (combined 25.5 times basal) and had an approximately additive effect in combination with Hp and both cytokines.

Conclusions: At least two pathways lead to IL-8 production in AGS cells: one involving PKC and another involving tyrosine kinase activity. These can be further enhanced by elevating intracellular calcium.

MUCOSAL SECRETION OF INTERLEUKIN-10 IN GASTRITIS.

K. Rodger, R.V. Healhey. St. James’s University Hospital, Leeds.

Background: Interleukin-10 (IL-10) is an 18kD cytokine with predominantly inhibitory actions, and is produced by a variety of cell types, in particular the Th2 subset of lymphocytes. This study aimed to establish whether IL-10 might play a role in gastric mucosal immune responses.

Methods: Antral biopsies (5x) were taken at endoscopy from 39 dyspeptic patients, and a serum sample collected. Patients taking NSAIDs or proton pump inhibitors were excluded. 2 biopsies were used for histology, and staining for HP, whilst 4 biopsies were incubated in tissue-culture chambers containing 1 ml RPMI-1640 culture medium at 37 °C for 24 hours. Culture supernatants were centrifuged, and stored at −20 °C. IL-10 was assayed in duplicate using a commercial ELISA (R&D Systems), corrected for biopsy weight, and the total IL-10 expressed as pg/24 hours. Serum HP IgG titres were determined by ELISA (to confirm infection).

Results: See table (*p<0.05):

<table>
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<tr>
<th>Histology</th>
<th>N</th>
<th>Mean IL-10</th>
</tr>
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<tbody>
<tr>
<td>Normal or reactive gastritis (HP negative)</td>
<td>17</td>
<td>59 pg/24 hr</td>
</tr>
<tr>
<td>Chronic gastritis (HP negative)</td>
<td>5</td>
<td>187 pg/24 hr</td>
</tr>
<tr>
<td>Chronic active gastritis (HP positive)</td>
<td>17</td>
<td>500 pg/24 hr</td>
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</table>

Conclusions: In-vitro mucosal IL-10 secretion was significantly elevated in patients with HP chronic gastritis compared to patients with HP-negative chronic gastritis (p<0.048), and those without chronic gastritis (p<0.003). Local production of this immunoregulatory cytokine in response to HP infection may serve to down regulate local inflammatory responses. Whilst this may be protective, limiting tissue damage caused by inflammation, IL-10 may also contribute towards failure of the host response to eliminate the infection. Further work is needed to establish the role of IL-10 in the immune response to HP, particularly in relation to the different disease manifestations.
Dietary Microparticles and Inflammation

RSJ Harvey, JJ Powell, CC Aisley, R. Wolinkscnott*, RPH Thompson.
Gastrointestinal Laboratory and Department of Immunology, The Rayne Institute, St Thomas’ Hospital, London SE1 7EH

INTRODUCTION: Sub-micron dietary inorganic particles are actively taken up from the intestinal lumen by specialized M cells and accumulate in underlying intestinal lymphoid aggregates. In the intestinal lumen particles can adsorb a range of biomolecules and such interactions may significantly alter the inflammatory properties of the particle and biomolecule.

AIMS: To demonstrate the adsorption of lipo polysaccharide (LPS) to titanium dioxide (TiO2); a ubiquitous food additive, and examine the effect of such particle modification on the activation of monocytes.

METHODS: Modified particles were formed by low energy sputter ion coating of fluorescein labelled LPS (100ng/ml) from E. coli 055:B5 with TiO2 5ng/ml in 4mM Ca2+ or deionized water. Particles were then separated by centrifugation and resuspension. LPS adsorption was determined by flow cytometry. Particle size was measured by photon correlation spectroscopy.

Table 1: Flow cytometry of LPS adsorption.

<table>
<thead>
<tr>
<th>% FITC+/+ particles</th>
<th>Particle size (nm)</th>
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<tbody>
<tr>
<td>TiO2</td>
<td>0</td>
</tr>
<tr>
<td>TiO2/LPS</td>
<td>1.5</td>
</tr>
<tr>
<td>TiO2/LPS +4.4Ca</td>
<td>31%</td>
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</table>

Separated blood mononuclear cells (5x10^6 cells/ml) from five normal volunteers were suspended in tissue culture medium containing modified particles (5ug/ml). After 24hr culture, supernatants were harvested for interleukin 1 assay (biossay) and the cells were labelled with monoclonal antibodies (the monococyte/macrophage markers CD14 + CD11c), and the activation marker CD25 for analysis by flow cytometry.

Results: Median (gmiB/GAPDH) for 24h culture, then centrifugation and resuspension. LPS adsorption was determined by flow cytometry. Particle size was measured by photon correlation spectroscopy.

Table 2: Flow cytometry of LPS adsorption.

<table>
<thead>
<tr>
<th>RESULTS (Mean±SEM)</th>
<th>Activated monocytes (CD25+)</th>
<th>Interleukin 1 (pg/ml)</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>5.5 ± 3.2</td>
<td>0</td>
</tr>
<tr>
<td>TiO2/LPS</td>
<td>13.9 ± 8.8</td>
<td>0</td>
</tr>
<tr>
<td>TiO2/LPS +4.4Ca</td>
<td>32.7 ± 14.4</td>
<td>450 ± 174</td>
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</table>

Conclusions: Titanium dioxide forms microaggregates with LPS and Ca+ ions which activate monocytes and stimulate the production of the pro-inflammatory cytokine interleukin 1. Similar processes in the intestine may be a mechanism of antigen delivery capable of generating mucosal hypersensitivity.

Cell/molecular biology T124-T130

Human Spasmylocytic Polypeptide Stimulation of Colorectal Cancer Cell Migration

Involves modulation of epithelial cadherin function: Preliminary observations.

Offi T, Karayannakis A, Playford R, Pignatelli M, Kmietow J. Departments of Histopathology and Colorectal Surgery, Royal Postgraduate Medical School, London

Four colorectal carcinoma cell lines were established in monolayer culture and in collagen gel. The lines were: HT29(E-cad negative) SW1112(E-cad positive), LS174T(E-cad negative) and an E-cad transfectant of LS174T, LSBAT7. Immunocytochemical staining extent for E-cad, E-cad and its cytoplasmic regulatory proteins alpha, beta and gamma catenins was estimated and cell migration in collagen gel measured by linear colony number before and after addition of 100mg/ml of recombinant hSP.

hSP was not constitutively expressed in monolayer culture or in collagen gel. Addition of hSP stimulated cell migration in SW1112 cells (10 colonies), in HT29 cells (20 colonies), LSBAT7 (10 colonies), but not in LS174T cells (10 colony). Expression of E-cad and the catenins in all 4 cell lines was not changed by addition of hSP. The motogenic effect of hSP in colorectal cancer cell lines may involve modification of E-cad function; the relevance of this in vitro finding with colorectal cancer stage and grade in vivo is being studied.