nuclear protein import. Further studies are needed to assess the functional importance of its glycosylation with sialylTF and to assess its potential as a target for cancer therapy.

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ENDOTHELIN-3 BLOCKADE PROMOTES PREMATURE NEURONAL DIFFERENTIATION IN AN ORGAN CULTURE MODEL OF HIRSCHSPRUNG DISEASE

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Aims: Pharmacological blockade of endothelin-3 (ET3) in an embryonic mouse gut culture system results in incomplete migration of enteric neural crest cells (NCC) along the gut and thus distal colonic aganglionosis. We aimed to determine the mechanisms controlling migration by analysing effects of endothelin blockade on NCC proliferation, differentiation, and apoptosis.

Methods: Gut was dissected from day 11.5 mouse embryos and transferred into culture with or without BQ788, a specific blocker for ET3's receptor (the ET_B receptor). NCC proliferation and apoptosis were determined by BrdU incorporation and TUNEL reactions respectively after 24h culture. NCC differentiation was determined using nitric oxide synthase (NOS) immunoreactivity after 24-72h culture.

Results: ET3 blockade had no effect on either proliferation or apoptosis of enteric NCC. After 72h, numerous NOS-positive neurons were identified in gut cultured with BQ788, but not in controls, and this was associated with distal colonic aganglionosis.

Conclusions: ET3 prevents enteric NCC differentiation rather than influencing proliferation or apoptosis. Thus, in the absence of ET3, migrating enteric NCC differentiate prematurely into non-migratory neurons and distal aganglionosis (Hirschsprung disease) results.

270 EPIDERMAL GROWTH FACTOR, ITS RECEPTOR AND THE ROLE OF GANGLIOSIDES G_{M1} AND G_{M3}

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Endocytosis is a process whereby eukaryotic cells take up extracellular material by a variety of different mechanisms. These endocytic functions are involved in the regulation of cell surface receptor expression, maintenance of cell polarity, cholesterol homeostasis and a host of other physiological processes. In this investigation we look specifically at receptor mediated endocytosis (RME), a pathway used for the internalisation of ligand/receptor complexes, in this case epidermal growth factor (EGF) and its receptor (EGFr). Gangliosides are necessary for the efficient functioning of this process and this study looks at the relationship between EGFr and gangliosides G_{M1} and G_{M3} in four oesophageal cell lines, and the impact of gangliosides on RME. We have also quantified the amounts of EGFr and gangliosides in the cell lines.

Background: EGF binds tightly to its receptor, a 175 kd single transmembrane protein, on the cell surface. Once bound, activation of specific protein kinases takes place inducing a number of cellular responses. Many gangliosides have been identified on the basis of their carbohydrate structure that is mainly expressed on the outer surface of the plasma membrane. They play an essential role in a variety of cellular responses such as cell adhesion, signal transduction, cell growth and differentiation.

Methods: Four oesophageal cell lines were investigated in tissue culture, two squamous cell carcinoma (SCC) and two adenocarcinoma (AC) cell lines. Cells were incubated with fluorescent labels, harvested for FACSscan® analyses or slide fixed for confocal microscopy.

Results: Co-localisation of EGFr and ganglioside on the cell membrane; cell lines with greater expression of EGFr also have greater amounts of ganglioside; increased levels of EGFr are present in SCC compared to that seen in AC; greater amounts of ganglioside are present in SCC than AC; G_{M3} inhibits the mode of action of EGF in SCC but not in AC.

Conclusion: These results provide us with information on the location of both EGFr and gangliosides on the cell membrane. Their co-localisation suggests that a co-operative relationship exists between

them. This evidence indicates that gangliosides are directly involved in the signalling mechanism that is induced when EGF binds to EGFr, a tenet supported by the inhibitory aspect of G_{M3} on one effect of EGF.

Immunology/Infection/ Inflammation Posters: 271-287

271 INCREASED EXPRESSION OF HUMAN β-DEFENSINS (hBD) 1 AND 2 IN *H. PYLORI*-POSITIVE AND -NEGATIVE GASTRITIS

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Introduction: *H. pylori* is the causative agent of most cases of chronic superficial gastritis in humans and a risk factor for the development of peptic ulcer disease and gastric cancer. Our understanding of the early host response to *H. pylori* infection is limited. We and others have previously shown increased expression of the anti-microbial peptide hBD2 by gastric epithelial cell lines in response to *H. pylori* infection. In the present study we investigated the mRNA and protein expression of hBD1 and hBD2 in *H. pylori*-positive and -negative gastric biopsy samples.

Methods: Gastric biopsies were obtained from patients with *H. pylori*-positive (n=11) and *H. pylori*-negative (n=5) gastritis and control subjects (n=8). hBD1 and hBD2 mRNA was quantified by quantitative and semi-quantitative RT-PCR. Immunohistochemistry was performed on archival gastric paraffin-blocked samples from patients with *H. pylori*- and non-*H. pylori*-related gastritis.

Results: A marked increase in hBD2 mRNA expression was observed in both groups of gastritis compared to control tissue (*H. pylori*-positive, p=0.006; *H. pylori*-negative, p=0.02). The constitutive hBD1 mRNA expression observed in control tissue was also upregulated (*H. pylori*-positive p=0.035; negative group p=0.01). A parallel increase was also observed in hBD1 and hBD2 peptides with the positive staining confined to the surface epithelium.

Conclusion: This is the first *in vivo* study showing increased expression of hBD1 and hBD2 in *H. pylori*-positive and -negative gastritis. These results suggest an important role for these peptides in the innate host defence during inflammatory episodes and infection.

272 CD44 REGULATION AND FUNCTION IN ULCERATIVE COLITIS

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We have shown that CD44v6 and CD44v3, variant isoforms of the cell surface glycoprotein CD44, are expressed on the baso-lateral surface of colonic epithelial cells in Ulcerative Colitis (UC) but not in other forms of colonic inflammation including colonic Crohn's Disease (CCD). The regulation of enhanced CD44 expression and the function of CD44 in UC have not been explained. CD44 is known to act as an adhesion molecule, to play a role in lymphocyte activation and to aid lymphocyte homing by binding and sequestering cytokines. Using FACS we have investigated the role of cytokines in inducing the expression of CD44v6 and v3 on the colonic cancer epithelial cell line HT-29. Using a novel lymphocyte adhesion assay we have gone on to investigate the role of CD44v6 and v3 in activated peripheral blood (aPBL) and gut lamina propria lymphocytes (LPL).

Results: IL-4 and IL-13 induced CD44v6 and v3 on HT-29 but IFN-γ, TNF-α, IL-1, 2, 6, 7, 8, and 10 did not. Time course experiments showed that expression was maximal at 12 hours. Co-incubation of HT-29 and IL-4 with hydrocortisone, TNF-α, IFN-γ, IL-2 and 10 resulted in down regulation of CD44v6 and v3. Using a novel adhesion assay we studied the adherence of aPBL and LPL to HT-29 monolayers pre-treated with IL-4 to induce CD44v6 and v3 expression. Monolayers of HT-29 cells were cultured in 96 well plates in the presence of medium alone or medium supplemented with IL-4 to induce CD44v6 expression in test wells. Fluorescently labelled PBL or LPL were added to the test and control wells, allowed

to adhere and then washed off. The effect of CD44 expression on PBL and LPL adhesion to HT-29 cells was determined by comparing the residual fluorescence in the presence or absence of CD44 induction. CD44 variant expression resulted in 2–3 fold increase in lymphocyte adhesion. This effect on adhesion could be blocked by pre-treating the HT-29 IL-4 wells with monoclonal antibodies known to block CD44 mediated adhesion.

Summary: Expression of CD44v6 and v3 on colonic epithelial cells is induced by IL-4. This effect can be competed out by TNF- α , IFN- γ , IL-2 and 10 and by hydrocortisone. CD44v6 and v3 mediate adhesion of aPBL and LPL to colonic epithelial cells. This effect can be blocked by antibodies to CD44 that may have a therapeutic role in UC.

273 FISH OIL SUBSTITUTED ELEMENTAL DIET IN ULCERATIVE COLITIS

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Introduction: Elemental diet is an effective anti-inflammatory agent in Crohn's disease but not in ulcerative colitis (UC). We have recently shown that elemental diet increases the IL1-receptor antagonist/IL1 β ratio in Crohn's disease, but not in ulcerative colitis *in vitro*. The lipid component of elemental diet is derived from coconut oil, canola oil and safflower oil. We have now investigated the effect of substituting fish oil (EF) for coconut oil, canola oil and safflower oil in elemental diet (EAA) in ulcerative colitis *in vitro*.

Methods: Colonoscopic rectosigmoid biopsies in six patients with UC were incubated in an organ culture model for 24 hours, by diluting elemental diet-fish oil (EF) or EAA (E028, SHS, UK) with modified Waymouth's complete medium (MB705/1) in dilutions of 1:20, 1:10, 1:5. Control was media alone without any added EF or EAA. The tissue viability was assessed by adding Bromodeoxyuridine (BrdU) to the culture fluid. The *in-vitro* uptake was confirmed by further immunohistochemical processing of the tissue and confirmation of BrdU labelled cells. The supernatant was collected after 24h and frozen at -70°C for immunoassay. ELISA was performed for pro-inflammatory (IL-1 β) and anti-inflammatory (IL-1ra) cytokines in the stored supernatants.

Results: Incubation of the organ culture specimens for 24 hours with EAA in 1:20, 1:10 and 1:5 dilutions did not increase the IL1ra/IL1 β ratio. In contrast, the IL1ra/IL1 β ratio increased when organ culture specimens were incubated for 24 hours with EF. The median IL1ra/IL1 β ratio (range) was 8.14 (2.67–17.67) when incubated with media alone. This increased to 46.42 (2.2–127.3) with 1:20 EF (p=0.02), to 18.2 (13.6–215.4) with 1:10 EF (p=0.01) and to 43.1 (18.9–111.7) with 1:5 EF (p=0.04), (Mann-Whitney test).

Conclusion: Fish oil substituted elemental diet increases the anti-inflammatory to pro-inflammatory cytokine balance *in vitro*, unlike standard elemental diet in ulcerative colitis. Clinical trials may be warranted if palatability problems can be overcome. This study corroborates a recent *in vivo* study showing reduction of disease activity in distal proctocolitis by n-3 PUFA administration.

274 MORPHOMETRIC ANALYSIS OF MACROPHAGES CONTAINING INORGANIC PARTICLES IN INFLAMMATORY BOWEL DISEASE

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Introduction: We have recently shown that microparticles can be transported across the intestinal epithelium. Such uptake of microparticles is more avid in inflamed epithelium compared to normal epithelium. We describe the presence and distribution of such particles within macrophages in IBD affected and normal intestine and nature of such particles.

Methods: Paraffin embedded sections from 12 surgically resected intestine (4 Crohn's disease, 4 ulcerative colitis and 4 normal from unaffected segment of cancer) were stained with H & E and cells with particles were located. In confocal laser scanning microscope (LSM) non-biological nature of particles was determined. Electron spectroscopic imaging with X-ray microanalysis (EDX) with transmission electron microscopy (TEM) was done to determine the composition

of the particles. Macrophages were detected in three step immunoperoxidase method using monoclonal antibody for CD68 antigen. Area of macrophages was measured under color detection system in image analysis (25 fields at x 400).

Results: Most macrophages in IBD, both Crohn's and UC contained inorganic particles which are non-biological. In contrast only a few particles were seen in one normal tissue. The particles were exclusively located in lysosomes within the cytoplasm of the macrophages. EDX analysis with TEM showed these particles to consist of titanium, silicon and aluminum. The area of tissue (μm^2) occupied by CD68 stained macrophages was significantly higher in IBD affected tissues compared to normal tissues [mean $\pm$ SE: Crohn's (858 $\pm$ 57), UC (582 $\pm$ 29) and normal (308 $\pm$ 20), p<0.01]. Proportion of macrophages (mean $\pm$ SE) in the tissues was: Crohn's (6.5 $\pm$ 0.4), UC (4.4 $\pm$ 0.2 and normal (2.3 $\pm$ 0.15), p<0.01.

Conclusion: IBD affected tissues contained particles of titanium, silicon and aluminum. This study confirms previous reports on presence of similar particulate material in IBD tissue and clearly shows that particles are exclusively present within the cytoplasm of macrophages. This study for the first time shows that macrophage area was increased in IBD-affected tissues, especially in Crohn's disease. Whether the presence of particles activates the macrophages to liberate pro-inflammatory cytokines and perpetuate inflammation requires further study.

275 GUT BACTERIA AND ULCERATIVE COLITIS—THE ENEMY WITHIN

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Introduction: Although ulcerative colitis (UC) appears to be the results of unrestrained inflammatory response, the antigen (s) which initiates and perpetuates this chronic, relapsing disorder remain uncertain. It has been suggested that ubiquitous luminal bacterial products are somehow involved in the pathogenesis of this disease. Furthermore, compelling evidence has been provided that not the mere presence of bacteria per se that induces colitis, but certain bacteria are needed, such as *Bacteroides* spp. We tested this hypothesis by using human model of patients with ileal pouch-anal anastomosis (IPAA, these patients virtually have no colon) and with active UC with recently developed physiologically representative method. We have also assessed the biological potentials of the bacterial products by using peripheral blood mononuclear cells (PBMC).

Methods: 19 patients with IPAA [10 with normally functioning pouch (NFP), median age 39 (28–73) and 9 patients with pouchitis, median age 36 (28–74) and 11 patients with active UC, median age 29 (19–66) as well as 12 age-matched healthy controls were investigated. All patients and controls underwent whole gut lavage (WGL) using polyethylene glycol-based solution drunk at a standard rate of 1litre/h. The first clear effluent was collected and processed. IgA antibody against *B. fragilis* and a 'cocktail' antigen [(Endotoxin core antigens were prepared from a range of Gram-negative bacteria (*E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Salmonella typhi*)] were measured by ELISA. TNF- α and IL-8 production were assessed by bioassay and radio-immunoassay respectively.

Results: The median value of IgA EndoCAB (antibody against endotoxin core antigens) in UC patients were significantly higher than that of controls and NFP (p<0.004 and p<0.003 respectively). Likewise, IgA EndoCAB concentration was significantly higher in pouchitis than that of NFP (p<0.008). The IgA antibody against *B. fragilis* in UC and pouchitis were significantly higher than that of NFP (p<0.04 and p<0.02). Both groups of bacterial antigens induces considerable amount of TNF- α and IL-8 in PBMC.

Conclusion: The study confirms that there is an unrestrained immune response against luminal bacterial antigens in the gut mucosa in patients with UC. The luminal antigens can induce inflammatory cytokines. Breakdown of tolerance towards anaerobic Gm-ve colonic bacteria may be a pathogenic mechanism of UC.

276 REGULATION OF CHEMOTAXIS BY ALPHA CHEMOKINES AND THEIR RECEPTORS IS ABNORMAL IN ULCERATIVE COLITIS

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Introduction: Alpha chemokines are molecules produced by activated gastrointestinal mucosa, vascular endothelium and circulating leukocytes which regulate chemotaxis of leukocytes into areas of inflammation. This study examines the contribution of alpha chemokines and their receptors to chemotaxis in patients with ulcerative colitis (UC).

Methods: The cellular expression of alpha chemokine receptors CXCR1 and CXCR2 on circulating leukocytes was measured by flow cytometry using conjugated monoclonal antibodies. An in-vitro chemotaxis assay was used to quantify the effects of alpha chemokines produced by UC patients and healthy volunteers, together with the effects of CXCR1 and CXCR2 blockade by purified monoclonal antibodies.

Results: Polymorph expression of CXCR1 and CXCR2 in UC patients (n=32) was significantly lower than in controls (n=15) but similar in lymphocytes and monocytes. UC patients with inactive disease had no significant difference in receptor expression compared to those with active disease ($p>0.1$). Interleukin-8 was found to be the most potent chemokine for all groups, but was significantly less active in UC than in controls ($p<0.02$). Chemotaxis mediated by interleukin-8, GRO-alpha and ENA-78 was significantly reduced by CXCR1 and CXCR2 blockade (67/42/25% and 35/48/38% respectively).

Conclusions: Patients with UC demonstrate reduced expression of alpha chemokine receptors on polymorphs and this does not appear to be a consequence of disease activity. Furthermore, the response to IL-8 is attenuated in UC patients. Therapeutic inhibition of CXCR receptors in ulcerative colitis warrants further investigation.

277 ALPHA-DEFENSIN EXPRESSION IN HUMAN JEJUNUM VARIES IN HIV INFECTION AND TROPICAL ENTEROPATHY

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Introduction: Paneth cells secrete α -defensins, endogenous antimicrobial peptides, but the extent to which expression varies in human populations or disease states is unknown. We set out to determine whether expression of human defensins (HD) 5 and 6 varies with HIV or parasitic infection or correlates with mucosal structure or function in jejunal biopsies from 84 adults participating in an ongoing study of tropical enteropathy in a poor urban community in Lusaka, Zambia. Approval for the studies was obtained from two ethics committees.

Methods: HD5 and HD6 mRNA expression was evaluated by quantitative RT-PCR, and peptide expression by immunohistochemistry using anti-HD5 kindly provided by Drs. Ganz and Porter (UCI, Irvine, CA). Mucosal morphometry was performed on well-orientated formalin-fixed sections.

Results: mRNA expression (log transcripts per μ g total RNA) varied from undetectable to 7. HD5 (but not HD6) mRNA was lower in HIV seropositive adults (median 5.0, interquartile range 0-6.1) than in HIV seronegative adults (5.7, 4.9-6.9; $p=0.01$). HIV seropositive adults were also more likely to have undetectable HD5 mRNA than seronegative adults (OR 4.0, 95%CI 1.4-1.2; $p=0.008$). HD5 mRNA (but not HD6) was inversely correlated with both villous height and epithelial surface area by rank correlation ($\rho=-0.41$, $p=0.003$) but only in HIV seronegative adults. Correlation between HD5 and HD6 expression increased with increasing grade of enteropathy ($\rho=0.04$ in minimal but $\rho=0.85$ in severe enteropathy). Expression did not differ in the presence or absence of parasitic infection. By immunohistochemistry peptide levels varied less between samples and no correlation was found with mRNA expression.

Discussion: HD5 expression is altered in enteropathy and HIV infection, but the regulatory mechanism(s) remain to be identified.

278 INTERLEUKIN-10 AND TGF- β 1 REGULATE INTERFERON- γ -MEDIATED INHIBITION OF CRYPTOSPORIDIUM PARVUM DEVELOPMENT IN ENTEROCYTES

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Introduction: Activation of macrophages by interferon (IFN)- γ to kill intracellular pathogens is down-regulated by interleukin (IL)-10,

transforming growth factor (TGF)- β 1 and IL-4. IFN- γ also activates enterocytes to inhibit development of intracellular pathogens, but the mechanisms regulating this are poorly understood. This investigation examined the modulatory effect of IL-4, IL-10 and TGF- β 1 on IFN- γ -mediated inhibition of development of *Cryptosporidium parvum*.

Methods: The human enterocyte cell line, HT 29, was grown to confluence in 24-well plates at 37°C. Human recombinant IFN- γ with or without either IL-4, IL-10 or TGF- β 1 was added to cell cultures starting 24h before infection (-24h) with *C. parvum*; alternatively, IFN- γ was added at -48h and the modulatory cytokine introduced at -24h, or vice versa. Twenty-four hours after infection with oocysts the monolayers were fixed with methanol and stained with Giemsa so that numbers of intracellular parasites could be determined microscopically.

Results: Treatment of enterocytes with IFN- γ starting 24h or 48h before infection inhibited parasite reproduction by 60-70%. Addition of TGF- β 1 or IL-10 to cell cultures at the same time as, but not 24h after, IFN- γ reduced the inhibitory activity of IFN- γ in a dose-dependent manner. In contrast, IL-4 had no significant effect on IFN- γ activity.

Discussion: These results suggest that IL-10 and TGF- β 1, but not IL-4, may be important in down-regulation of Th1-mediated activation of gut epithelium infected by intracellular pathogens. Once activated by IFN- γ , however, enterocytes may be refractory to modulation by IL-10 or TGF- β 1.

279 MAP KINASE INHIBITION REVERSES CRYPTOSPORIDIUM PARVUM INDUCED IL-8 SECRETION BY ENTEROCYTES

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Introduction: *C. parvum* is a major cause of diarrhoea world-wide. The parasite has previously been shown to induce the expression of the C-X-C chemokine IL-8 by infected enterocytes. Mitogen-activated protein (MAP) kinase activation is important in signalling the regulation of inflammatory gene expression in response to a variety of external stimuli including pathogens. We determined the role of MAP kinases in mediating infection and in modulating the induction of IL-8 by enterocytes infected with *C. parvum* using selective inhibitors in our established in vitro model.

Methods: Cells of the intestinal cell line HCT 8 were grown to confluence in 24 well plates. Cells were treated with either non-selective MAP kinase inhibitor apigenin (Apig, 25 μ M), selective p38 kinase inhibitor SB203580 (20 μ M), selective MEK inhibitor PD98059 (50 μ M), the inactive related compound SB202474 (negative control, 20 μ M), or medium alone with or without DMSO. Treated cells were infected with *C. parvum* (0.25-2x10⁶ oocysts or purified sporozoites/well) and incubated 24-48h. Infection was quantified by immunofluorescence assay, using an anti-*C. parvum* polyclonal antibody 24h after parasite inoculation. ELISA was used to quantify IL-8 protein in supernatants collected 48h after parasite inoculation.

Results: *C. parvum* induced an inoculum-dependent increase in IL-8 in serum-starved HCT 8 cells, with a peak at 48h and 1x10⁶ sporozoites (2629.5 pg/mL, $\pm$ 90.1) compared with untreated controls (1334.1 pg/mL, $\pm$ 21.6). MAP kinase inhibitors did not modify infection of HCT 8 cells. However MAP kinase inhibition was found to partially reverse *C. parvum* induced IL-8 induction (Apig 83.2 % $\pm$ 0.4, $p<0.01$; SB203580 50.0 % $\pm$ 4.2, $p<0.05$; PD98059 35.7 % $\pm$ 4.9, $p<0.05$).

Discussion: Our findings indicate that MAP kinase inhibition may partially reverse the induction of IL-8 by *C. parvum* in vitro. In contrast MAP kinase inhibition had no action on parasite infection of enterocytes. We hypothesise that MAP kinases function as upstream mediators of NF- κ B activation and resultant IL-8 gene induction following *C. parvum* enterocyte infection.

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280 PRO-INFLAMMATORY CYTOKINES INHIBIT CRYPTOSPORIDIUM PARVUM INFECTION OF ENTEROCYTES

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Introduction: We have previously shown IFN- γ may directly inhibit *C. parvum* infection of human intestinal cell lines. In this study we have examined the action of other pro-inflammatory cytokines, IL-1 β , TNF- α , IFN- α , IL-15, and the anti-inflammatory cytokine TGF- β 1 on parasite infection of enterocytes in our established in vitro model. These cytokines may all be produced by enterocytes and are therefore potential mediators of the innate mucosal host immune response to infection.

Methods: HT29 intestinal cells grown on coverslips were infected with *C. parvum* oocysts (2×10^5 oocysts/well) as previously described. Cells were pre-treated with IFN- α (0.01–1 ng/mL); IL-15 (1–100 ng/mL); IL-1 β (0.1 ng/mL); TNF- α (1 ng/mL); or TGF- β 1 (5 ng/mL) for 24 h prior to parasite inoculation. Infection was quantified by immunofluorescence assay, using an anti-*C. parvum* polyclonal antibody and Giemsa staining. Inhibition or enhancement of infection in the treated wells was calculated as percentage of infection in untreated controls.

Results: All of the pro-inflammatory cytokines assessed had an inhibitory effect on parasite infection. IFN α maximal inhibition was $35.3\% \pm 10.7$ (ANOVA $p < 0.001$). IL-15 maximal inhibition was $40.1\% \pm 8.1$ (ANOVA $p < 0.001$). IL-18 maximal inhibition was $31.1\% \pm 7.1$ (ANOVA $p < 0.001$). IL-1 β inhibition was $33.0\% \pm 8.1$ ($p < 0.001$). TNF- α inhibition was $61.0\% \pm 7.8$ ($p < 0.01$). In contrast, TGF- β 1 enhanced infection by $36.6\% \pm 7.8$ ($p < 0.05$).

Discussion: We have demonstrated that a range of pro-inflammatory cytokines may inhibit in vitro infection of intestinal epithelial cells by the parasite *C. parvum*. Production of these cytokines by the enterocyte offers a potential innate mechanism of mucosal immunity to infection.

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281 THE ROLE OF IL-12 AND IFN- γ IN IMMUNITY TO CITROBACTER RODENTIIUM INFECTION

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Enterohaemorrhagic (EHEC) and enteropathogenic (EPEC) *Escherichia coli* are important human pathogens. The formation of "attaching and effacing" (A/E) lesions on gut enterocytes is central to the pathogenesis of EHEC and EPEC, a feature shared with the non-invasive enteric mouse pathogen *Citrobacter rodentium*. Although *C. rodentium* is a non-invasive pathogen, previous studies (Higgins et al, *Infect Immun* 67:3031) have shown that infection is associated with a mucosal Th1 immune response. We have therefore examined the role of IL-12 and IFN- γ in host defense against *C. rodentium* infection. Following infection, IL-12p40 and IFN- γ deficient mice had higher numbers of *C. rodentium* in mucosal and systemic tissues than wild type mice, and 25% of the IL-12 deficient mice died. The striking features of the pathology in IL-12 deficient mice were firstly, patchy lesions present in the mucosa, with some areas showing a huge infiltrate of CD4+ cells and mucosal hyperplasia, and other areas with few CD4+ cells and no colonic hyperplasia; secondly bacteria were seen at the base of the glands in the IL-12 deficient mice compared to controls, which correlated with the absence of epithelial mouse β -defensin 3 expression. IFN- γ and TNF- α mRNA transcripts were also seen in the colon of IL-12 deficient mice although phosphorylated Stat4 and mature IL-18 were not increased. These studies are the first to show an important role for IL-12 and IFN- γ in limiting non-invasive bacterial infection of the colonic epithelium, and also demonstrate that Th1 activation in the gut wall can occur in the absence of IL-12 and IL-18.

282 URINALYSIS FOR INTERLEUKIN-8 IN THE NON-INVASIVE DIAGNOSIS OF ACUTE AND CHRONIC INTRA- AND EXTRA-ABDOMINAL INFLAMMATORY CONDITIONS

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Background: In preliminary studies, we have shown that interleukin-8 (IL-8) levels rise in patients with active inflammatory bowel disease. To explore its diagnostic potentials, we measured urinary IL-8 levels in a range of acute and chronic inflammatory conditions, in both adults and children.

Methods: IL-8 was measured by ELISA in random urine samples (1 ml each), carrying code numbers, and taken from 208 patients: 177 adults and 31 children. Active inflammation was diagnosed clinically, with the aid of acute phase responses and, where appropriate, using radiology, endoscopy, and histology.

Results: In adults, the median urinary IL-8 level was 75 pg/ml in those with active chronic inflammatory bowel disease ($n=25$) compared with 20 pg/ml in patients with inactive disease ($n=28$), and 9 pg/ml in controls ($n=14$; $P=0.001$). Patients with active chronic rheumatoid arthritis ($n=21$) had a median IL-8 level of 93 pg/ml, compared with 11 pg/ml in those with inactive arthritis ($n=22$; $P=0.001$). The median IL-8 value in subjects with non-specific abdominal pain or constipation ($n=20$) was 8 pg/ml compared with 107 pg/ml in patients with acute cholecystitis ($n=15$; $P<0.0001$), 127 pg/ml in those with appendicitis ($n=8$; $P=0.0001$), 584 pg/ml in patients with urinary tract infection ($n=12$; $P<0.0001$), and 191 pg/ml in those with diverticulitis or gastroenteritis ($n=12$; $P=0.0001$). Children with non-specific conditions ($n=10$) had a median IL-8 level of 10 pg/ml compared with 199 pg/ml in those with non-viral inflammation/ infection ($n=12$; $P=0.0001$), and 7 pg/ml in patients with viral upper respiratory tract infection ($n=9$; $P=0.7$). In the study group as a whole, urinary IL-8 values correlated positively with peripheral blood white cell count, erythrocyte sedimentation rate, and C-reactive protein.

Conclusions: Urinary IL-8 measurement helps in the non-invasive assessment of active inflammation. It is simple and has the potential to be used by both clinicians and patients.

Acknowledgements: The work is protected by an international patent application.

283 INCREASED SUSCEPTIBILITY OF TNFRP55 DEFICIENT MICE TO CITROBACTER RODENTIIUM (MOUSE EPEC) INFECTION

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Infection of mice with the intestinal bacterial pathogen *Citrobacter rodentium* (murine EPEC) results in colonic mucosal hyperplasia and a local Th1 inflammatory response similar to that seen in mouse models of IBD. In these latter models, and in patients with Crohn's disease, neutralisation of TNF- α is of therapeutic benefit. Since there is no information on the role of TNF- α in either immunity to non-invasive bacterial pathogens such as *Citrobacter*, nor in the role of TNF- α in infectious colitis, we have investigated *Citrobacter* infection in TNFRp55^{-/-} mice. In wild-type C57BL/6 mice *Citrobacter* infection does not alter weight gain, despite the presence of a high bacterial burden in the colon. In contrast, in TNFRp55^{-/-} mice, there are higher bacterial burdens, but weight loss only occurs late in infection. Bacteria are however cleared showing that TNF- α is not needed for protective anti-bacterial immunity. The most striking feature of infection in TNFRp55^{-/-} mice however is the markedly enhanced pathology, with increased mucosal weight and thickness, increased T cell infiltrate and a markedly greater mucosal Th1 response. The enhanced bacterial burden is probably due to the increased mucosal surface area in the TNFRp55^{-/-} mice. To help explain the enhanced Th1 associated immunopathology we examined IL-12 expression. IL-12 p40 transcripts were markedly elevated in *Citrobacter* infected TNFRp55^{-/-} mice and this was associated with enhanced STAT4 phosphorylation. TNF- α may therefore play a protective role in the intestine in response to bacterial infection by down-regulating tissue-damaging Th1 responses.

284 IN VITRO MODELLING OF INTESTINAL BACTERIAL COMMUNITIES COLONISING MUCIN

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The large intestine is covered with a protective mucus coating that harbours bacterial populations believed to be distinct from those in

the gut lumen. While little is known of their composition or metabolic activities, they are likely to play an important role in mucus breakdown.

Aims: To investigate the formation of mucin biofilm communities, and to study physiological and enzymological factors that enable mucin degrading species to compete for substrates in biofilms.

Methods: Colonisation of mucin gels by faecal bacteria was studied using a two-stage chemostat, simulating conditions of nutrient stress characteristic of the proximal (vessel 1) and distal (vessel 2) colons. Establishment of bacterial communities in the gels was investigated using selective culture methods, scanning electron microscopy and confocal laser scanning microscopy, in association with fluorescently-labelled 16S rRNA oligonucleotide probes. Gel samples were also taken for analysis of mucin degrading enzymes and measurements of residual mucin sugars (Dionex).

Results: Mucin gels were rapidly colonised by heterogeneous bacterial populations, especially members of the *B. fragilis* group, enterobacteria and clostridia. Planktonic anaerobe: facultative anaerobe ratios were 5000:1, whereas in the mucin gels, this dropped to 3:1 (vessel 1) and 6:1 (vessel 2), which is similar to the rectal mucosa. N-Acetyl neuraminidase, β -galactosidase and N-acetyl β -glucosaminidase rapidly appeared in the gels. Apart from N-acetylgalactosamine in vessel 1, all mucin oligosaccharide sugars were digested by colonic bacteria. Destruction of mucin was most extensive by organisms growing under strongly carbon-limited conditions in vessel 2. Galactose and N-acetyl glucosamine were utilized most effectively. Acetate and butyrate production by planktonic bacteria in vessels 1 and 2 was more rapid than in microbial biofilm communities, which mainly produced acetate.

Conclusions: Intestinal bacterial populations colonising mucin surfaces in the chemostats were shown to be phylogenetically and metabolically distinct from their planktonic counterparts. *In vitro* modelling of the microbiota in this way has wider applicability, for example, in studying the effects of antibiotics and drugs on gut biofilms.

285 MIGRATION OF PRECURSORS OF INTRAEPITHELIAL LYMPHOCYTES (IELs) FROM HUMAN COLONIC LAMINA PROPRIA (LP)

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Background: Together with epithelial cells, IELs represent the first cells of intestinal defence. IELs express the integrins $\alpha E\beta 7$ and a restricted repertoire of $\alpha\beta$ T-cell receptors (TCR), as shown by restricted variable (V) region usage of the TCR β -chain (V β). In addition to V region, TCR $\alpha\beta$ genes also consist of diversity (D), joining (J), and constant (C) regions. The epithelial basement membrane contains many discrete pores through which IEL precursors migrate *in vivo* from the lamina propria (LP) into the epithelium. Using an *ex-vivo* model, we have investigated the migration of IEL precursors from the colonic lamina propria.

Methods: Human colonic mucosal samples (n=11) were denuded of the epithelium and placed in medium. T cells migrating out of the lamina propria (via basement membrane pores) were collected after 1 h and 16 h culture and studied by FACS and quantitative PCR (for ratios of αE :TCR α mRNA). TCR V β region usage was investigated by spectratyping using 24 family-specific PCRs. The number of peaks (reflecting the sizes of VDJ regions in the PCR products) for each member of TCR V β family was determined. Data are expressed as mean ($\pm$ SEM).

Results: A greater proportion of CD8⁺ T cells expressed αE integrin than CD4⁺ cells [0–1 h culture: 38.1($\pm$ 5.4)% vs 13.8($\pm$ 5.4)%, $p=0.005$ and 1–16 h culture: 38.0($\pm$ 5.1)% vs 11.8($\pm$ 2.7)%, $p<0.001$]. The ratio of αE :TCR α mRNA transcripts in cells migrating out of the lamina propria over 0–1 h culture of denuded mucosal samples was significantly greater than in cells collected after the 1–16 h culture period [8.7($\pm$ 2.2) vs 1.8($\pm$ 0.5); $p=0.024$]. Amplified TCR V β transcripts of cells migrating over 0–1 h culture period showed fewer peaks than those migrating over 1–16 h period [3.1($\pm$ 0.3) vs 6.4($\pm$ 0.3); $p<0.001$].

Conclusions: Following loss of the surface epithelium, majority of the αE -expressing T cells migrating out of the human colonic lamina propria are CD8⁺. Cells migrating out of the lamina propria during the 1st h of culture express higher levels of αE transcripts and show a restricted repertoire of TCR V β . These cells are likely to be IEL precursors.

286 A ROLE FOR PLATELET ACTIVATING FACTOR AND TUMOR NECROSIS FACTOR IN THE REGULATION OF MMP'S IN THE GUT; A POTENTIAL EXPERIMENTAL MODEL FOR NECROTISING ENTEROCOLITIS

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Introduction: Necrotising enterocolitis is a major cause of morbidity and mortality among pre-term infants, occurring in up to 10% at all neonatal intensive care unit admissions. The symptomatic spectrum of NEC can range from mild intestinal ischemia to severe bowel infarction and necrosis in addition to a range of systemic symptoms. Many studies have implicated MMPs in tissue injury in the gut, in particular stromelysin-1 (MMP-3) which is produced in excess by cytokine and mediator activated stromal cells. Several pro-inflammatory mediators and cytokines [Tumor necrosis factor alpha (TNF α), interleukin (IL)-6 and platelet activating factor (PAF) and IL-1 β and IL-8], have been detected in blood and in surgical specimens from infants with NEC.

Aim: To set up a human model for NEC and to ascertain the effects for PAF and TNF α on the expression levels of key MMPs.

Materials and methods: Human foetal gut explants and a foetal gut derived myofibroblast cell line were cultured and stimulated with PAF-16 and TNF α and the culture supernatants analysed by western blotting.

Results: Under basal conditions the expression of MMPs 1, 3 and 9 are low. Upon the addition of PAF the levels of MMPs 1, 3 and 9 increase moderately in a dose dependent fashion with the highest level of expression being achieved under physiological conditions (18ng/ml). When a physiological dose of TNF α alone is added, the levels of MMP 1, 3 and 9 increase beyond the levels observed with PAF alone. However, PAF plus TNF α , synergise to dramatically increase the levels of MMP expression beyond the levels observed when either factor is used alone. In contrast, MMP-2 and TIMPs remain constitutively expressed. This profile has been observed in both foetal gut explants and myofibroblast cells.

Conclusions: PAF and TNF α act synergistically as potent mediators of MMP expression. This suggests a potential role for MMPs in disease-associated tissue destruction in an experimental model of NEC.

287 EXPRESSION AND FUNCTIONS OF PURINERGIC RECEPTOR P2X₁ IN COLONIC MACROPHAGES AND T CELLS FROM NORMAL AND INFLAMMATORY BOWEL DISEASE MUCOSA

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Introduction: P2X₁ receptor is an ATP-gated ion channel which can form large membrane pores and has been implicated in cytokine release and apoptosis in inflammatory cells. We therefore investigated its expression, activation and inhibition in normal and diseased colonic macrophages and T cells.

Methods: Expression of P2X₁ receptor mRNA was studied by RT-PCR. Concentration-dependent changes in pore formation (ethidium bromide influx), apoptosis (annexin V staining) and IL-1 β release (sandwich ELISA) were used as functional assays. Ultrastructural changes were studied by transmission electron microscopy.

Results: P2X₁ receptor mRNA was present in all colonic macrophages and T cells studied. Pore formation and apoptosis were induced by purinergic ligands, with a potency order typical for P2X₁ receptor agonists (BzATP>ATP>2MeSATP). The concentration of ATP to induce 50% apoptosis was 84 μ M for macrophages and 20 μ M for T cells respectively (n=4). ATP-induced apoptosis could be specifically inhibited both by apyrase hydrolysis or by the P2X₁ receptor antagonist, oxidised ATP, KN-62 and by P2 pan receptor antagonist PPADS, but not the P2Y receptor antagonist. Functional evidence of apoptosis and pore formation was associated with loss of cell membrane continuity. In LPS-primed macrophages, ATP caused caspase-1 activation and rapid concentration-dependent release of mature IL-1 β , which was inhibited by o-ATP (IC₅₀=30 μ M). The proportion of unstimulated macrophages and T cells recovered from ulcerative colitis (72%, n=5) and Crohn's disease (70%, n=4) tissues

that were apoptotic was significantly higher than for normal (32%, $n=8$), and not further increased by BzATP. o-ATP reduced the spontaneous IL-1 β release in IBD samples.

Conclusions: Expression and functional data support the presence of P2X₇ receptor on colonic mucosal macrophages and T lymphocytes. This may play a role in the immunopathology of inflammatory bowel diseases.

Small Bowel Posters: 288–307

288 IGA ANTIBODY TO TISSUE TRANSGLUTAMINASE IS A HIGHLY SENSITIVE PREDICTOR OF ADULT ONSET COELIAC DISEASE IN CLINICAL PRACTICE

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Introduction: Anti-endomysial antibodies have high specificity (90%) for the presence of coeliac disease, but there are ethical and cost issues associated with using monkey oesophagus for clinical tests. Tissue transglutaminase (tTG) is the main autoantigen recognised by endomysial antibodies and commercial tests have recently become available to measure tTG in serum.

Aim: To establish the diagnostic sensitivity, specificity, positive and negative predictive value of IgA anti-tTG in adult clinical practice.

Methods: The study comprised 48 patients (mean age 52 years: range 20–86, 31 females) undergoing investigation for possible coeliac disease. Patients with known coeliac disease were excluded from study. Serum IgA anti-tTG antibody titres were measured quantitatively by an enzyme-linked immunosorbent assay (QuantaLite tTG, Inova Diagnostics, Ca, USA). Samples were classified as negative (<20 units), weakly positive (20–30) or moderate to strongly positive (>30 units).

Results: Duodenal biopsies were normal in 40 patients and untreated coeliac disease was diagnosed in the remaining 8 cases. All 8 patients with coeliac disease had moderate to strongly positive anti-tTG antibody titres. In addition, 9 of 40 patients without coeliac disease were anti-tTG antibody titre positive (5 weakly positive, 4 moderate to strongly positive). Overall, IgA antitissue tTG had a diagnostic sensitivity, specificity, positive and negative predictive value of 100%, 78%, 47% and 100% respectively.

Conclusion: IgA antibody to tissue transglutaminase appears to be a highly sensitive predictor of adult onset coeliac disease in clinical practice. The relatively high false positive rates for tTG in this material requires further study.

289 IS A RAISED INTRAEPITHELIAL COUNT WITH NORMAL VILLOUS ARCHITECTURE CLINICALLY SIGNIFICANT?

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The significance of an increase in intraepithelial lymphocytes (IEL) within an otherwise normal duodenal biopsy remains uncertain. Although it may represent latent coeliac disease, there is very little information available about cases that do not have gluten-sensitive enteropathy.

Methods: A consecutive series of routine endoscopic duodenal biopsies from August '98–July '99 reported by a single pathologist was collected to assess the incidence of such a finding. Clinical details and indications for biopsy were examined in all patients. Those biopsies initially reported to have subjectively increased IELs with normal villi, together with a control group reported as normal, were reassessed by the pathologist blinded to the original report. IEL counts in the study group were compared to controls.

Results: Of 625 patients biopsied in the 12 month period, 507 were reported as normal, 104 had a specific duodenal pathology and 14 (2.2%) had increased IEL with normal villi. The ages, sex ratio and clinical indications for biopsy were similar in the latter 14 when compared to the 507 patients with a normal biopsy. Enumeration of the IEL/100 epithelial cells confirmed an increase in all 14 patients

(range 26–46/100). In the normal control group, the mean was 12.4, giving an upper limit of normal as 21.5 (mean + 2SD). Of these 14, 3 were positive for coeliac antibodies. The other diagnoses included chronic liver disease (3), colonic tumours (2; 1 benign, 1 malignant), irritable bowel syndrome (2), chronic pancreatitis (1) and unexplained iron deficiency anaemia (3).

Conclusions: A raised IEL count with normal villi is not uncommon (2.2%). There is still no clear explanation for its finding in many patients. As it may represent latent coeliac disease, long term follow up studies are required, especially in those with positive coeliac antibodies.

290 COELIAC DISEASE: EVIDENCE OF ADDITIONAL SUSCEPTIBILITY WHEN THE SECOND HAPLOTYPE IS A DR7 DQ2.1 OR A DR3 DQ2.2 IN DR3 DQ2.2 POSITIVE SUBJECTS

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Introduction: 90% of UK coeliac patients carry at least one DR3 DQA1*0501 DQB1*0201 (DQ2.2) haplotype. Patients without this haplotype nearly always carry either a DR4 DQ8 or encode the DQA1*0501 DQB1*0201 heterodimer in *trans* on DR5 and DR7 haplotypes. There is some evidence that in patients with a DR3 DQ2.2 haplotype increased susceptibility is conferred by the second haplotype being either a DR7 DQA1*0201 DQB1*0202 (DQ2.1) or a DR3 DQ2.2.

Aims: To assess whether a DR7 DQ2.1 haplotype conveys additional coeliac disease susceptibility in subjects with one DR3 DQ2.2 haplotype, and to compare this susceptibility to DR3 DQ2.2 homozygotes.

Methods: HLA haplotypes were determined in 58 family pedigrees each containing at least 2 coeliac patients. A PCR-SSP method was used to type for DR7, DR3, DQA1*0201, DQA1*0501, DQB1*02 in 37 families, with 21 families only having typing for DQA1 and DQB1. In these 21 families two microsatellites, and in 29 families 1 flanking microsatellite flanking the HLA region were assessed following PCR using ABI 373 and 377 sequencers.

Results: 42 coeliac patients were identified where, having inherited a DR3 DQ2.2 from one parent, the other parent was heterozygous for DR7 DQ2.1 (in some families more than one coeliac patient was used). In 28 of these 42 cases the DR7 DQ2.1 haplotype was transmitted to the patient ($p<0.05$, McNemars test, Yates correction) demonstrating an additive effect of this haplotype on coeliac disease risk in DR3 DQ2.2 positive patients. In 18 of these cases the other parent was heterozygous for DR7 DQ2.1 with DR3 DQ2.2. In 11 of these 18 cases the DR7 haplotype was transmitted to the patient ($p=NS$). Compared to DR3 DQ2.2 negative subjects, the haplotype relative risk for DR3/DR3 homozygotes, DR3/DR7 heterozygotes and DR3/x* heterozygotes were 36.1, 35.1, 10.7 respectively. (*where x is not DR3 or DR7).

Conclusion: A DR7 DQ2.1 haplotype conveys additional risk for coeliac disease in DR3 DQ2.2 positive patients. This additional risk was no greater than for DR3 DQ2.2 homozygotes consistent with the additional effect of a DR7 DQ2.1 haplotype being a dosage effect of DQB1*02.

291 FOLLOW-UP LINKAGE STUDY OF COELIAC DISEASE: FURTHER EVIDENCE FOR THE EXISTENCE OF A SUSCEPTIBILITY LOCUS ON CHROMOSOME 11p11

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The susceptibility to coeliac disease has a strong genetic component, as demonstrated by a 10% disease risk in first degree relatives, 30–50% concordance in HLA identical siblings, and a 70–100% concordance in monozygotic twins. The contribution of the HLA region to this genetic component has been well described but it is clear that loci outside this region must also contribute to susceptibility. Two previous genome wide linkage studies using the affected sib pair