Neoplasia, cell biology, immunology

W45

AGE RELATED DEVELOPMENT OF MUCIN GLYCOPROTEINS IN THE COLONIC MUCUS GEL LAYER OF CHILDREN

A. Aslam, R. D. Spicer, *A. P. Corfield , (introduced by B. Warren)
Department of Paediatric Surgery, Bristol Children's Hospital, Bristol, U.K. and *University Department of Medicine Labs., Bristol, U.K.

Mucin glycoproteins are the major component of the colonic mucus gel layer and interact with pathogens performing a protective function. We studied developmental changes in mucin glycoproteins in normal children from birth to the age of 15 years. We used colonic mucosal organ culture with radioactive mucin precursors [35S]-sulphate and [3H]-glucosamine in dual isotope labeling experiments. After 24 hours culture the secreted and cellular mucus fractions were collected. Mucins were purified by gel filtration and the ratio of incorporation ([35S]/[3H]) was measured. The turnover of radioactive precursors was quantified by relating it to the DNA content of the mucosa ([35S] or [3H] per μg DNA). The total mucin in each sample was assessed by reactivity with Wheat germ aggultinin and the turnover of total mucin was quantified. Mucins were tested against 5 anti-mucin antibodies and 2 lectins. Three age groups were used guided by usual weaning and dietary changes, and had mucosal biopsies taken for culture. They were 0-3 months (9 subjects), 3 months-3 years (20), and 3-15 years (12). The ratio of incorporation showed a statistically significant trend of increase with age in both secreted and cellular fractions. The turnover of both [35S] and [3H] and total mucin was significantly higher in the youngest age group in both fractions. This was followed by a sharp drop in turnover in the 3 month-3 year group followed by a gradual rise to a level about half that of early infancy in older children. The antibody 91.9H (which detects human sulphated mucin) showed a significant trend towards increasing sulphation in the older age groups and this correlates with the increasing [35S] ratio, especially as the reactivity with the antibody PR3A5 (which detects di- and tri-O-acetylated sialic acids) did not change. The other antibodies also showed no significant differences. This is the first time developmentally regulated changes in the colonic mucus glycoproteins have been identified and characterized.

W46

SOMATOSTATIN AND GASTRIN RELEASING PEPTIDE EXPRESSION IN THE DEVELOPING HUMAN STOMACH.

EJ Kelly and SJ Newell, Regional Neonatal Unit, St. James's University Hospital, Leeds.

In adults Somatostatin (SS) and Gastrin Releasing Peptide (GRP) have opposite effects on gastric acid production. In the developing stomach the role of these mediators is unknown. We have previously shown that the developing human stomach expresses the Hydrogen-Potassium ATPase from 13 weeks gestation and even the most immature infants are capable of producing, and maintaining, a low intra-gastric pH from within 24 hours of birth (Arch Dis Child 1994, 71:F136-41). To further evaluate the roles of SS and GRP in the developing human it is first necessary to know the ontogeny of these peptides.

Serial sections from 30 fetal (14-38 weeks gestation) and 5 infant (2-21 weeks) stomachs were stained using standard histological techniques using antibodies against SS and GRP and viewed by two independent observers.

SS was found in the developing human stomach from 14 weeks gestation onwards, the D cells increasing in numbers throughout gestation, and located predominantly in the antrum. GRP immunoreactivity was not found in any of the fetal or infant stomachs examined.

This is the first study to comment upon the ontogeny of SS and GRP in the human stomach. Further studies need to be performed to elucidate their specific roles in maintaining the achlorhydria associated with birth and the rapid production of a low intra-gastric pH which is seen in even the most immature infant.

W47 W47–W58

A PROSPECTIVE RANDOMISED TRIAL OF CIMETIDINE THERAPY IN GASTRIC CANCER: AN INTERIM REPORT.

Hallissey, MT, Dunn, JA, Baker, PG, Davis, JG, Billingham,LJ, Fielding, JWL, Langman, MJ for the British Stomach Cancer Group.
CRC Trials Unit, University of Birmingham, Birmingham, UK

Cimetidine has been reported to improve the survival of patients with gastric cancer at all stages of disease. The aim of the fourth British Stomach Cancer Group trial was to assess the survival benefit of adjuvant cimetidine in gastric cancer.

The study is a randomised double-blind trial comparing cimetidine at 2 dose levels, 800 or 400mg twice daily with matching placebos. Eligible patients had biopsy proven gastric adenocarcinoma, with any stage of disease, were considered fit to enter the trial (life expectancy > 3 months) and were able to give informed consent. The study recruited 442 patients between February 1990 and March 1995 from 59 consultants in 39 hospitals throughout the UK. Analysis has been undertaken on an intention-to-treat basis. There is now 12 months follow up on all patients with a median follow up of 41 months.

The treatment allocation was balanced for sex, age, and stage. The median age of the patients in the study population is 68 years, (range 23 - 88 years). The male to female ratio was 1:2.5. The majority of patients had stage III (31%) or stage IV (46%) disease, with 63 (15%) stage II and 36 patients (8%) stage I.

The trial results demonstrate no survival benefit for cimetidine when comparing the survival for all patients receiving cimetidine against placebo (χ^2 =1.77, p=0.18). When adjustment is made for dose (400 vs 800mg), stage (I/II, III, IV), age (<68, 68+) and sex, there is no survival benefit for the use of cimetidine. The median survival is 11 months for the cimetidine group compared to 12 months for the placebo group. The median survival within the cimetidine group is 11 months (95% CI 6 - 16 months) for 800mg BD and 12 months (95% CI 8 - 14) for 400mg BD.

The results of this trial do not support the use of cimetidine as an adjuvant therapy in gastric cancer. The need to continue trials of therapy in gastric cancer remains.

W48

LIVER RESECTION PROMOTES THE GROWTH OF DISTANT TUMOUR IN THE RAT.

ME Caplin[1], T Morris[2], RE Pounder[1], AP Dhillon[3], SA Watson[2]. Departments of Academic Medicine[1] and Histopathology[3], Royal Free Hospital School of Medicine, London. Cancer Studies Unit[2], University of Nottingham, Nottingham.

Introduction: Liver resection results in the release of a number of trophic agents which contribute to liver regeneration. It has previously been shown in an animal model that liver resection promotes the growth of intrahepatic metastases. **Aim:** To determine whether liver resection promotes the growth of distant tumour. **Method:** 20 BD1X rats (10 female and 10 male) were each given an injection of 1 x 10^5 cells of a colon cancer cell line DHDK12 into the muscle layer of the abdominal wall. After 1 week 10 rats (5 female = group I; and 5 male = group II) underwent 70% liver resection. As a control 10 rats (5 female = group III; and 5 male group = IV) underwent sham operation with laparotomy only. At 3 weeks post surgery all rats were terminated and growth of abdominal wall tumour was assessed. **Results:** There was a significant increase in the tumour growth between female rats group I vs. group III with tumour weight 0.354mg (SD 0.07) vs. 0.24mg (SD0.07) a difference of 47.5% p<0.05 and tumour cross-sectional area 87.7mm^3 vs. 74.0mm^3 a difference of 18.5% p<0.03. There was a smaller difference in tumour growth in males group II vs. group IV with tumour weight 0.41mg(SD0.09) vs. 0.39mg (SD0.09) p=NS and tumour cross-sectional area 104.7mm^3 (SD43.5) and 77.3mm^3 (SD14.6) p=NS. Overall in rats undergoing liver resection vs. sham operation there was an increase in tumour weight 0.38(SD0.08) vs. 0.32(SD0.11) a difference of 21% p=0.06 and a significant increase in tumour cross sectional area 121.7mm^3 vs. 80.65mm^3 a difference of 51% p<0.05. **Conclusion:** There is an increase in the growth of distant tumour following liver resection in the rat. The difference in tumour growth compared to sham operated rats was more significant in the female rats. Those growth factors involved in liver regeneration may well promote the growth of more distant tumour. This study additionally raises the question of female sex hormones upregulating the effect of these growth factors.

W49

FEEDING AND DISTENSION-INDUCED EXPRESSION OF *c-fos* IN RAT GASTRIC CORPUS MUCOSA

P-J.M.Noble,A.D. Higham,R.Dimaline,G.J.Dockray.
Physiological Laboratory, University of Liverpool, Liverpool, U.K.

Background : Expression of the immediate early gene *c-fos* is increased in rat gastric corpus within 30 minutes of feeding. This increase occurs -in part- in neurons of the myenteric plexus but it is not clear if there is also increased expression in the mucosa.

Aim: To establish if luminal stimuli control *c-fos* expression in gastric corpus mucosa and to determine the factors involved.

Methods: Abundance of *c-fos* mRNA in rat gastric corpus mucosa was analysed by Northern blot hybridisation using cRNA probes. Gastric mucosal *c-fos* was measured in rats fasted for 48hrs and refed for various times. The role of myenteric neurons in the mucosal *c-fos* response was examined in rats in which the anterior face of the corpus had been denervated by serosal application of 0.5%(w/v) benzalkonium chloride (BAC). The role of gastric distension was examined by distending the stomach in gastric fistula rats to a pressure of 5 cm water with either 2% methyl cellulose buffered at pH 6.0 or 50mM HCl in 2% methyl cellulose.

Results: In rats refed following a 48hr fast, corpus mucosal *c-fos* mRNA increased fourfold within 30 minutes ($p<0.01$,ANOVA,n=6) and returned to basal levels in 3 hrs.The refeeding response was the same in BAC treated (i.e.denervated) and untreated regions of the corpus. Gastric distension with 50mM HCl in 2% methyl cellulose elicited a 1.6 fold rise in *c-fos* ($p<0.05$,ANOVA,n=6) while distension with methyl cellulose buffered at pH 6.0 produced a small non-significant elevation.

Conclusion: i) Food increases abundance of *c-fos* mRNA in gastric mucosa.ii) This response does not require the myenteric plexus. iii) Mild noxious stimuli of the mucosa by non-nutritive distension with acid also stimulates *c-fos* expression.

W50

EXTRA-PANCREATIC ION TRANSPORT IN CHRONIC PANCREATITIS.

NM Sharer, J Painter, D Marland, and JM Braganza.
Pancreato-Biliary Service, Royal Infirmary, Manchester, UK. M13 9WL.

This study in patients with chronic pancreatitis aimed to find out whether possession of a mutant cystic fibrosis gene is associated with alterations in sweat electrolytes and/or nasal transmembrane potential difference (NPD). Of 126 consecutive patients with chronic pancreatitis, 18 (14%) carried a mutation ($p < 0.0001$ vs. local controls). 11 of these patients underwent pilocarpine iontophoresis and NPD measurements. Results were compared with those from 13 age-matched patients without a mutation and 15 healthy controls.

	Chronic pancreatitis		Controls
	mutant *CFTR*	normal *CFTR*	
sweat tests			
sweat [Na^+] mmol/l	54 (34.5 - 104)*	46 (25 - 90.5)*	30 (22 - 66)
sweat [Cl^-] mmol/l	34 (19 - 80)*	30.5 (10 - 75.5)*	18 (10 - 39)
NPD (-mV)			
baseline (B)	-10.41 (1.34)*	-8.14 (0.49)	-7.18 (0.43)
ΔA–B, amiloride (A)	-4.76 (1.11)	-3.30 (0.58)	-3.03 (0.67)
ΔC-A, low Cl^- (C)	-11.05 (5.08)	-13.07 (5.23)	-17.03 (6.16)
ΔI-C, isoprenaline (I)	-4.48 (0.81)	-6.14 (1.76)	-6.76 (1.24)

[Sweat data as median (range) and NPD as mean (SEM), * $p < 0.05$ versus controls].

The patients as a whole had CF-like anomalies of sweat electrolytes but there was no difference between those with and or without a *CFTR* mutation. Although baseline values of NPD were lower in *CFTR* carriers the response to agents that perturb ion transport was comparable to controls. These data confirm previous reports of increased sweat electrolytes in patients with chronic pancreatitis, but suggest that this and NPD abnormalities are not entirely explained by possession of a *CFTR* mutation.

W51

THE ROLE OF TRANSFORMING GROWTH FACTOR ALPHA ON KININ STIMULATED ION TRANSPORT AND BARRIER FUNCTION IN HUMAN COLONIC EPITHELIAL CELLS

Beltinger J, Stack WA, Hawkey CJ. Divison of Gastroenterology, University Hospital, Nottingham NG7 2UH, UK.

Introduction: Enhanced PG synthesis has recently been shown after activation of EGF receptors with TGFa on the basolateral cell surface of HCA-7 cells. Bradykinin has been shown to stimulate electrogenic chloride secretion both by a Ca^{2+} dependant pathway and via eicosanoid production which can be attenuated by a cyclooxygenase inhibitor. We used the colonic epithelial cell line HCA-7, colony 29 to study the modulatory effect of TGFa on transepithelial resistance and bradykinin induced chloride secretion and to show the possible interaction with local PG synthesis.

Methods: HCA-7 cells were grown in DMEM (10%FCS), seeded on Snapwell filters and formed confluent monolayers within 10-12 days. Cells were either treated with TGFa (10ng/ml) for 24h in the presence or absence of NS398 (10^{-5}M), a specific cyclooxygenase-2 (COX-2) inhibitor, added two hours before removing cells from the wells. The filters were placed into an Ussing chamber bathed in oxygenated Krebs-Henseleit solution and voltage clamped by continous application of a short circuit current (SCC). Basal SCC($\mu A/cm^2$) and resistance(Ωcm^2) was measured and after an equilibration period bradykinin (BK) (10^{-6}M) was added. All drugs were added to the basolateral side of the monolayer. Data are expressed as mean±SEM.

Results: TGFa 10ng/ml significantly increased baseline resistance (control 138±9.9 vs TGFa 169±16.2Ωcm^2, n=20, p=0.03). The COX-2 inhibitor NS398 did not affect baseline resistance of controls and in TGFa pretreated monolayers (TGFa 169±16.2 vs TGFa+NS398 178±17.2Ωcm^2, n=13, p≠s). Δ SCC due to BK was attenuated by TGFa (control 13.3±2.7 vs 9.2±1.8$\mu A/cm^2$,n=11, p=0.03,). Δ SCC to BK was further reduced by NS398 in untreated controls and TGFa pretreated cells (control vs control+NS398:13.3±2.7 vs 6.6±1.4$\mu A/cm^2$, n=6, p=0.04; TGFa vs TGFa+NS398: 9.2±1.8 vs 4.4±1.3$\mu A/cm^2$, n=6, p=0.03).

Conclusion: These data provide evidence of the regulatory role of growth factors on transepithelial resistance and BK stimulated ion transport in intestinal epithelial cells with a central role of PG synthesis which may have important implications in pathogenic conditions such as inflammatory bowel disease.

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W52

INCREASED HUMAN GASTRIC ENDOTHELIAL CELL COX-2 EXPRESSION DURING ANGIOGENESIS.

MA Hull, JL Brough, CJ Hawkey. Division of Gastroenterology, University Hospital, Nottingham, NG7 2UH.

INTRODUCTION: Angiogenesis plays an important role in gastric ulcer healing. Prostaglandins (PGs) promote angiogenesis *in vivo* and non-steroidal anti-inflammatory drugs (NSAIDs) reduce vascularity of gastric ulcer granulation tissue and delay healing. However, it is not known whether gastric ulcer angiogenesis is associated with changes in endothelial cell cyclooxygenase (COX) expression. Therefore we investigated COX expression during *in vitro* angiogenesis using human gastric endothelial (HuGE) cells which were obtained using an immunomagnetic separation technique developed in our laboratory.

METHODS: HuGE cells were isolated from normal gastric mucosa using anti-PECAM-1 antibody-coated Dynabeads. HuGE cells were cultured routinely on 1% gelatin in Medium 199 + 30% FCS + 90 µg/ml heparin + 40 µg/ml ECGS until plating onto basement membrane matrix (Matrigel) or addition of 1 µM phorbol 12, 13 dibutyrate (PdBu). COX-1 and COX-2 expression were investigated by RT-PCR, western blotting and indirect immunofluorescence studies. PGE2 levels in cell-conditioned medium were measured by ELISA.

RESULTS: PdBu induced formation of cell extensions and "ring" structures in HuGE cells at 4 hours which was associated with increased COX-2 expression and PGE2 production. COX-2 was localized predominantly in the nuclear envelope. At 24 hours COX-2 expression had declined. There was no change in COX-1 expression after addition of PdBu. Formation of "tube-like" structures by HuGE cells on Matrigel was associated with an increase in COX-2 (and COX-1) mRNA expression and PGE2 production which was maintained at 24 hours.

CONCLUSION: In this model of angiogenesis, HuGE cell differentiation (formation of "tube-like" structures) rather than proliferation was associated with induction of COX-2 and COX-1 expression. This process may be impaired by NSAIDs during gastric ulcer healing. These findings suggest that specific COX-2 inhibitors may also impair angiogenesis and delay gastric ulcer healing.

W53

DETECTING ANTIBODIES FROM THE GUT: FAECES VERSUS GUT PERFUSATE

Nicholas M Croft, Anne Ferguson
Dept of Medicine (WGH), University of Edinburgh, Western General Hospital, Edinburgh EH4 2XU

Background: Specific antibodies are not always detectable in faecal specimens when present in whole gut lavage fluid (WGLF) (a gut perfusion technique) collected concurrently. The possibility that the presence of diarrhoea improves the detection and recovery of antibodies led us to study 14 adults with ileostomies. **Methods:** Ileostomy effluent (IE) was collected for 24 hours. A fresh aliquot of IE was collected, extracted and centrifuged. The supernatant was filtered, processed, and stored at -70°C. Whole gut lavage was performed at 1 litre per hour and the resulting specimens handled as above. Anti-ovalbumin IgA and IgM (Ova-A & M), anti-gliadin IgA and anti-cholera toxin IgA and IgM were assayed by ELISA. The output in ileostomy effluent was estimated by multiplying the concentration in IE by the 24 hour output, output in WGLF was the product of the concentration and the rate of the lavage. **Results:** Detection of antibodies was similar in WGLF and ileostomy extracts. The % recovery of

Antibody	Detectable in:		Median
	WGLF	IE	Recovery
Ova-A	14/14	14/14	12.3
Ova-M	11/14	9/14	9.3
Gliadin-A	14/14	12/14	10.1
Cholera Toxin-A	14/14	14/14	19
Cholera Toxin-M	14/14	14/14	11.1

antibodies (expressed as 100 x output in IE/output in WGLF) was between 9 and 19 %. For ovalbumin-IgA, the most readily detectable antibody, there was an increased recovery in 8 ileostomy specimens with a water content above 92% (median recovery 18.3 %) compared with 6 specimens below 92% (median recovery 7.3%, $p<0.05$). **Discussion:** These results show that IE is comparable to WGLF for the detection of antibodies, however measured output is very much less in IE. There is an increased recovery of ovalbumin-A in specimens with higher water content. **Conclusions:** Diarrhoea may be used to detect the presence/absence of antibodies but not for estimating daily outputs. However comparing diarrhoeal and non-diarrhoeal faecal material is an inappropriate way to study antibody responses in the gut.

W54

SEROLOGICAL MARKERS FOR COELIAC DISEASE: CHANGES WITH TIME AND RELATION TO ENTEROPATHY

SD Johnston, RGP Watson, SA McMillan, AHG Love.
Department of Medicine, Royal Victoria Hospital, and Regional Immunology Laboratory, Belfast.

Coeliac disease is associated with antibodies to gliadin, endomysium and reticulin. The significance of elevated antibody titres detected by population screening is unclear.

AIM: To determine the stability over time of IgA-antibodies to gliadin (AGA), endomysium (EMA) and reticulin (ARA) and their relation to enteropathy.

METHODS: Fifty-one subjects from a large representative population survey, the Belfast MONICA Project (1992), detected as having AGA, EMA and/or ARA, were followed up after 3 years. Jejunal biopsies were carried out endoscopically by means of a Crosby capsule. AGA was detected by ELISA; EMA and ARA by indirect immunofluorescence. Titres of AGA > 100AU were taken as significant.

RESULTS: Overall 28 (55%) of 51 subjects with significant titres of at least one serological marker at initial screening developed negative serology at 3-year follow-up. Thirteen of 23 subjects with persistent serological markers had enteropathy. All 9 subjects with EMA at follow-up had enteropathy, in contrast to 7 of 14 subjects with a persistently significant titre of AGA and 1 of 3 with persistent ARA. Serological determination was repeated for all blood samples and AGA titres were correlated by Spearman Correlation Coefficient ($r=0.94$; $p<0.001$) and EMA and ARA by kappa statistic ($r=0.87$; $p<0.001$ and $r=0.91$; $p<0.001$ respectively).

CONCLUSIONS: Serological markers for coeliac disease detected by population screening are often transient and, in the majority, do not indicate enteropathy. EMA is a better indicator of enteropathy than AGA or ARA in population screening. Persistent serological markers for coeliac disease in relatively healthy subjects are an indication for jejunal biopsy since at least half of them are likely to have enteropathy.

W55

ENTEROPATHOGENIC E. COLI STIMULATE TYROSINE KINASES IN GASTRIC AND COLONIC EPITHELIAL CELLS.

Hyde GM and Jewell DP. Gastroenterology Unit, Radcliffe Infirmary, Oxford.

Tyrosine kinases are an integral part of cellular signal transduction pathways and are involved in the control of cell proliferation, differentiation and gene transcription. E. coli have been implicated in the pathogenesis of ulcerative colitis, with increased numbers of adhesive and invasive strains present in patients compared to healthy controls. Enteropathogenic E. Coli (EPEC) are known to tyrosine phosphorylate a 90kD protein in HeLa cells. We have demonstrated stimulation of tyrosine kinases by EPEC in $CaCO_2$ (a colonic cell line) and AGS (a gastric cell line). **Method** The strains of EPEC, E2348/69 and cfm mutant 14.2.1(1) were cultured on MacConkey agar and then inoculated into Luria Bertani broth to increase the yield. The cells were cultured at 37°C, 95% O_2, 5% CO_2 and then subcultured into petri dishes at a density of $2\text{-}5x10^6$ cells/ml, the day before the experiment. 100µl of bacterial broth, either E2348/69or 14.2.1(1) was added to each petri dish (optical density 1.5 at λ = 600nm) in duplicate. Control plates contained cells alone. Infection proceeded for three hours at 37°C, 95% O_2, 5% CO_2. Cells were washed with PBS and lysis buffer added. The resulting solution was centrifuged and the supernatant run on a 1% SDS PAGE electrophoresis minigel. The proteins were subsequently transferred to a nitrocellulose membrane by Western blotting. The membranes were immunostained with an antiphosphotyrosine antibody as primary antibody and a peroxidase conjugated rabbit anti mouse immunoglobulin as secondary antibody and then developed in an Amersham ECL camera. **Results** In both $CaCO_2$ and AGS, enteropathogenic E. Coli E2348/69, but not cfm mutant 14.2.1(1) stimulated the production of a 90kD tyrosine phosphorylated protein, which was inhibited by genistein, a known inhibitor of tyrosine phosphorylation. **Conclusion** Stimulation of tyrosine kinases is a potential pathogenic mechanism for E. Coli as a candidate, causative bacterial agent in ulcerative colitis. Work investigating the effect of adhesive and non-adhesive E. Coli in $CaCO_2$ cells and well-defined genotypic strains of helicobacter pylori in AGS cells are underway.

W56

INVASION OF MOUSE PEYER'S PATCH M CELLS BY *YERSINIA PSEUDOTUBERCULOSIS*.

M. Ann Clark, Barry H. Hirst, Mark A. Jepson. Department of Physiological Sciences, University of Newcastle upon Tyne NE2 4HH.

The Peyer's patches appear to be the primary site of invasion of the orally transmitted enteropathogenic *Yersinia* species. This suggests that the specialised antigen sampling intestinal M cells in the overlying follicle associated epithelium (FAE) may provide the portal for *Yersinia* invasion, as has been shown for other pathogens, but to date attempts to elucidate the role of M cells in *Yersinia* invasion have been hindered by the lack of M cell markers. In the present study we have utilised the recently identified mouse M cell marker *Ulex europaeus 1* (UEA1) to investigate, by confocal laser scanning microscopy (CLSM), the role of M cells in the pathogenesis of *Y. pseudotuberculosis*: this technique permits analysis of bacterial invasion within extensive areas of FAE.

Peyer's patch-containing ligated intestinal loops were created in anaesthetised mice and inoculated with the wild type strain *Y. pseudotuberculosis* YPIII/pIB1. After 15-120 min incubation the Peyer's patches were harvested, fixed and dual stained for bacteria and M cells. Examination of the FAE by CLSM revealed that the majority of bacteria observed were associated with M cells. Bacteria were associated, either as single or, more frequently, as groups of bacteria, with M cells which were often situated in small clusters amongst regions of FAE containing M cells lacking bacteria. *Yersinia* invasion of M cells was observed after only 15 min incubation, but was more frequent at later time points: after 60 min, groups of bacteria were observed as deep as 20 µm below the M cell surface. Bacterial interaction with the M cells was accompanied by a redistribution of M cell-associated UEA1 staining around adherent/invaded bacteria.

Our studies demonstrate that M cells are the major site of *Y. pseudotuberculosis* invasion of murine intestinal Peyer's patches. This hypothesis supports previous data which demonstrate that M cells are a major site of invasion by a diverse range of pathogens. Elucidation of the mechanisms utilised by such pathogens to invade M cells may permit the development of effective disease control strategies based on prevention of M cell invasion.

Inflammatory bowel disease T59–T70

W57

INTERNATIONAL DIVERSITY IN THE MID-REGION OF THE *Helicobacter pylori* VACUOLATING CYTOTOXIN GENE, *vacA*.

JC Atherton, M Karita, G Gonzalez-Valencia, MR Morales, KC Ray, RM Peek, GI Perez-Perez, TL Cover, MJ Blaser. Vanderbilt University and VAMC, Nashville, TN, USA. (Intro. by CJ Hawkey).

In the US, *H. pylori vacA* shows allelic variation in the signal sequence (which may be type s1a, s1b or s2) and the mid-region (type m1 or m2). Previous PCR-based *vacA* mid-region typing classified most, but not all, Asian and South American strains tested as m1 or m2. We now sought to investigate *vacA* mid-region diversity further.

Methods: We studied 13 Japanese, 6 Chinese, 9 Thai, and 8 Peruvian *H. pylori* isolates. *cagA* was identified by colony hybridisation (CH), *vacA* signal sequence was typed by PCR, and *vacA* mid-region was typed proximally by CH and distally by PCR. Sections of the *vacA* mid-region from 8 strains were PCR-amplified, sequenced, and compared with known sequences from 8 other strains.

Results: Of the 36 Asian and South American strains studied, 35 were *cagA*$^+$ (the *cagA*$^-$ was Peruvian) and 35 were *vacA* s1a (1 *cagA*$^+$ Peruvian was s1b). *vacA* mid-regions from the 13 Japanese strains were not PCR-amplified by m1 or m2-specific primers, but hybridised weakly with an m1 probe. Sequence analysis of *vacA* from 1 Japanese strain revealed 91% nucleotide identity with the m1 probe but only 71% identity with the m2 probe. The previously equivocal Thai and Peruvian strains also had m1-like mid-region sequences. A Chinese strain was m1 in the proximal mid-region and m2 distally, showing a clear crossover site. Final mid-region types were: Japanese, all 13 m1; Chinese, 1 m1, 1 m1/m2, 4 m2; Thai, 3 m1, 6 m2; Peruvian 4 m1, 4 m2. Distal mid-region sequences of 16 strains, compared over 294 bp, clustered into 2 groups, m1 and m2. Nucleotide identity between m1 and m2 strains ranged from 73-78%. Within groups, m2 strains were less diverse than m1 strains (m2 range 94-99.7%, m1 88-99.3%, $p < 0.001$). Sequence analysis of 7 m1 and 3 m2 strains over 1.1kb proximally showed maintenance of clustering outside the 294bp region

Conclusions: These Asian and South American strains are similar in terms of *cagA* status and *vacA* s1a genotype, but fall into 2 *vacA* mid-region groups. m1 sequences are more diverse than m2, and thus may be phylogenetically older. The *vacA* sequence of 1 Chinese strain suggests recombination *in vivo* between m1 and m2 alleles.

T59

CHILDHOOD RISK FACTORS FOR IBD USING A TWIN CASE-CONTROL METHOD

Subhani J, Thompson N, Wakefield A, Ebrahim S, Pounder R
The Royal Free Hospital, Pond St., London NW3

Previous case-control studies of IBD have used hospital or community derived controls. There are obvious inherent biases in both methods, which also fail to control for genetic factors. Same-sex twin studies overcome both these flaws and are statistically more powerful.

Method: Each member of a registry of 175 twins-pairs, at least one of which had IBD, was independently sent validated questionnaires confirming their disease, zygosity and asking about childhood (age <16 years old) risk factors.

Results: In 130 cases both twins replied, 116 discordant for the disease, 80 of which were the same gender (47 monozygotic). These 80 pairs formed the case-control study.

Risk factor	Case	Control	Odd ratio (95% CI)
Measles	64	56	1.71 (0.8 - 3.8)
Mumps	38	43	0.78 (0.4 - 1.5)
Chickenpox	64	65	0.94 (0.4 - 2.2)
Asthma & Hayfever	8	13	0.57 (0.2 - 1.6)
Tonsillectomy	17	17	1.0 (0.4 - 2.3)
Appendicectomy	0	4	p=0.12
Pneumonia	3	7	0.41 (0.1 - 1.9)
Gastroenteritis > twin	22	5	5.69 (1.9 - 20) *
Exposure to animals > twin	16	3	6.42 (1.7 - 35) **

* p= 0.0007 ** p= 0.003

The inaccuracy of retrospectively collected data is a concern in these studies. This method allowed an assessment of its validity by checking the agreement of the twins answers, this ranged between 75-99%.

Conclusion: These preliminary results confirms the increased frequency of episodes of "gastroenteritis" and reveals an increased exposure to animals. This method overcomes many of the traditional problems of case-control studies. The greater degree of matching increases the statistical power and comparing answers provides an internal validation of the data.

W58

HELICOBACTER PYLORI (*H.PYLORI*) ANTIMICROBIAL RESISTANCE IN THE UK.

QN Karim*, RPH Logan† : the Glaxo Wellcome *H.pylori* Study Group.
* St Mary's Hospital, Paddington. †University Hospital, Nottingham.

Introduction: *H.pylori* antimicrobial susceptibility is an important determinant of the efficacy of eradication therapies[1]. The prevalence of antimicrobial resistance varies within the UK and may increase given the increased use of eradication therapy. This multicentre study assesses the prevalence and possible associations of *H.pylori* antimicrobial resistance.

Methods: *H.pylori* was isolated from antral biopsies of patients undergoing routine endoscopy and cultured according to standard microbiological methods. Antimicrobial resistance was determined using "E-tests" or disc tests (tinidazole only) with breakpoints defined by previous studies.

Results: *H.pylori* was isolated from 32% (1222/3823) of patients and antimicrobial susceptibility determined in 90% (1077/1222) of positive cultures.The prevalence of resistances (median + ranges) for the most widely used antimicrobials are:

Metronidazole	Tinidazole	Clarithromycin	Tetracycline
38.6 (14.6-65.2)	28.2 (7.2-41.5)	4.8 (1.3-12.5)	2.2 (0.7-6.3)

Tetracycline resistance was seen in 22 isolates. The prevalence of metronidazole resistance was greater in isolates from inner city centres (45.1%) compared with those in rural centres (17.7%). This difference was statistically significant (P<0.001). Patients less than 40 years of age showed greater resistance to metronidazole than patients over 40 years (P<0.001); resistance being more prevalent in the female population (P<0.001). Over a 2 year period, there has been no change in the prevalence of metronidazole or clarithromycin resistance. Multiple resistance was seen in approximately 5% of isolates.

Conclusion: The prevalence of antimicrobial resistance to *H.pylori* does not appear to be increasing but varies with location, gender and age; predictors of metronidazole resistance. In addition, multiple antimicrobial resistance seen in approximately 5 % of *H.pylori* positive isolates, underlines the importance of establishing local patterns of antimicrobial resistance and selecting appropriate eradication regimens.

Ref. 1: Penston JG. Aliment. Pharmacol. Ther 1994; 8:369-389

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FAMILIAL AGGREGATION AND CONCORDANCE IN CLINICAL CHARACTERISTICS IN CROHN'S DISEASE.

M. PEETERS, H. NEVENS, F. BAERT, M. HIELE, A.-M. DE MEYER, R. VLIETINCK, P. RUTGEERTS. Centre for Gastrointestinal Research, University of Leuven, B-3000 Belgium.

Background: Age adjusted risks are important for genetic counselling and modelling in Crohn's disease. Moreover, knowledge of concordance in disease characteristics is important to define phenotypic subtypes.

Aims: First, to determine familial occurrence and age adjusted risks in first degree relatives of Crohn's patients compared to controls. Second, to evaluate the agreement in disease characteristics within Crohn's families.

Methods: Crohn's patients (n=640) and controls (n=800) were questioned about familial occurrence of IBD in their first degree relatives. In addition agreement for age at diagnosis, initial disease location, disease behaviour and number of bowel resections was determined in 68 multiply-affected families and 100 unrelated Crohn's patients.

Results: Crohn's probands had a more frequent ($p<0.001$) positive family history for Crohn's disease (13.6%) than controls (1.1%). Risk estimates were significantly higher in relatives of patients compared to controls. The highest age adjusted risk for IBD was found in offspring (10.4%). Especially daughters of Crohn's patients showed an important IBD risk (12.6%). Parents were significantly ($p<0.001$) older at diagnosis than their offspring. Within generation age at diagnosis was very similar ($r=0.69$, $p<0.001$). The initial disease location showed a significant agreement within familial cases ($\kappa=0.285$; 0.057-0.512). Especially between siblings the agreement was striking ($\kappa=0.372$; 0.119-0.626).

Conclusions: Our large family study provides for the first time age adjusted risks for a European population. The clinical impression of familiality in disease characteristics was confirmed by using an objective measure of agreement, namely the κ-value.