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1. HELICOBACTER PYLORI GASTRITIS IS ASSOCIATED WITH EXPRESSION OF VARIANT FORMS OF CD44 ON GASTRIC EPITHELIAL CELLS: IMPLICATIONS FOR GASTRIC CANCER

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CD44 is a cell adhesion molecule which is involved in adherence of cells to extracellular matrix ligands such as hyaluronic acid. Numerous variants of CD44 are known to exist as a result of alternative splicing and post-translational modifications of the CD44 gene. Expression of CD44 variants has been noted in gastric, colorectal and breast cancers and lymphomas. It has been suggested that expression of CD44 variants (CD44v6 and CD44v9) by cancers could facilitate growth and metastasis of cancer cells. This is supported by evidence that expression of CD44 variants is associated with a poor prognosis in gastric cancer. The mechanism responsible for the expression of CD44 variants in cancers is uncertain. We investigated the role of Helicobacter pylori (HP) infection on the expression of CD44 variants on gastric epithelial cells and gastric lamina propria lymphocytes and compared with expression of HLA-class II antigens.

10 patients with HP gastritis and 8 patients with normal gastric mucosa were studied. HP infection was confirmed by the CLO test and histology. Antral biopsies were treated to separate gastric epithelial cells (EC) and intra epithelial lymphocytes (IEL). Monoclonal antibodies to CD44 (L3DI and D2.1), CD44v9, CD44v6, HLA-DR, HLA-DP, HLA-DQ were used and expression on LPL and gastric epithelial cells was determined by flow cytometric analysis.

CD44 expression was present at low levels on normal gastric EC. CD44, CD44v9 and D2.1 but not CD44v6 expression was significantly increased on gastric EC in HP chronic gastritis (HP+). CD44 expression in HP+ vs. normal antrum (HP-) (mean±s.e.m); 154±48 vs. 29±10 mfi (mean florescence intensity), $p<0.05$. D2.1 expression in HP+ vs. HP- antrum; 216 ±50 vs. 178±36 mfi, $p<0.05$. CD44v9 in HP+ vs. HP- antrum: 27±7 vs. 14±2 mfi, $p<0.05$. There was no significant difference in CD44 (167±34 vs. 146±19 mfi, p=ns) or D2.1 (216±50 vs. 178±36 mfi, p=ns) expression on gastric IELs in HP+ compared HP- normal antrum. Class II expression (DR, DP, but not DQ) was increased on gastric IELs and gastric epithelial cells of HP+ antrum compared to normal gastric mucosa. Gastric IEL DR expression: HP+ vs. HP-; 14±4 vs. 4±1 mfi, $p<0.05$, Gastric IEL DP expression: HP+ vs. HP-; 19±5 vs. 7±4 mfi, $p<0.05$. Gastric EC, DR expression: HP+ vs. HP-; 13±4 vs. 4±1 mfi, $p<0.05$. Gastric EC, DP expression: HP+ vs. HP-; 10±3 vs. 2±1 mfi, $p<0.05$.

Conclusion: H pylori may be responsible for epithelial CD44 expression in gastric cancer. The mechanism by which H pylori induces CD44v9 expression on gastric EC is uncertain but may be either a direct effect of H pylori or the result of the host immune response. Differences between epithelial CD44 and HLA class II expression noted in this study suggest that different cytokine profiles may be responsible for expression of class II compared to CD44.

2. ARGININE-INDUCED PANCREATITIS IS NOT MEDIATED BY NITRIC OXIDE

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An animal model of acute pancreatitis can be caused by intraperitoneal injection of arginine in rats. The mechanism of this is unknown but is either a direct toxic effect or may be by means of its metabolic product nitric oxide (NO). NO synthesis can be inhibited by administering l-NAME at the same time as the arginine. The role of NO in the lung is controversial - studies on lung damage following ischaemia-reperfusion injury implicate NO, but one study on caerulein-induced pancreatitis appears to show a beneficial effect .

35 adult male Sprague-Dawley rats were treated as follows: a) 5 rats were sacrificed for baseline histology; b) 10 rats had l-arginine (500 mgs/100g body weight) injected intra-peritoneally after serum amylase estimation; c) 10 rats had l-NAME (77.4 mgs/100g body weight) injected intra-peritoneally after serum amylase estimation; d) 10 rats had both arginine and l-NAME injected simultaneously. All were sacrificed after 24 hours for histological examination.

Arginine induced pancreatitis in 9 of the 10 rats. This was classified as mild in 8 cases; l-NAME on its own had no effect. Arginine plus l-NAME caused a moderate/severe pancreatitis in 7 and a mild pancreatitis in 3 ($p = 0.02$, χ^2 test). This was confirmed by the rise in serum amylase (1380 [735] vs 11145 [2330], mean [s.e.m.], $p = 0.002$, t test). Lung histology was normal in 2 of the arginine group but in 5 of those who also had l-NAME. The wet:dry lung ratio was significantly higher in the arginine group (5.1 [0.8] vs 4.2 [0.7], mean [SDI, $p = 0.01$, t test).

These results suggest that arginine induces pancreatitis by a direct effect and that NO is protective. However, as in ischaemia-reperfusion injury, NO appears involved in pancreatitis induced ARDS.

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3. A SHORT-DURATION, INEXPENSIVE REGIME TO ERADICATE HELICOBACTER PYLORI

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Eradication of H. pylori heals gastric and duodenal ulceration and dramatically reduces the long-term ulcer recurrence rate. However, only 10% of all peptic ulcer patients are treated with eradication therapy. Current eradication regimes have several disadvantages. Side-effects, frequent dosing, length and cost of treatment and poor compliance give rise to varying results and a reluctance to prescribe eradication therapy.

Two recent studies suggested that a one week course of omeprazole, metronidazole and clarithromycin was well tolerated and yields eradication rates of 92-100%.

The aim of the study was to access the efficacy of a new one week triple therapy regime for the eradication of H.pylori.

After routine diagnostic upper gastrointestinal endoscopy,

subjects with H. pylori gastritis were enrolled. Patients were classified as H. pylori positive if two of the following three tests were positive; histology, culture and rapid urease test. Suitable subjects received omeprazole 20mgs mane, clarithromycin 250mgs b.i.d., and metronidazole 400mgs b.i.d. for one week (total cost; IR £26.95. MIMS, July 1994). Eradication was assessed four weeks after treatment by repeat endoscopy and biopsy. Two subjects who did not attend for endoscopic follow-up had a 13C Urea breath test performed.

Forty subjects were enrolled (24 male and 14 female, mean age 44.8 years, range 17-83 years).

The regime was well tolerated; 9/40 (22.5%) reported mild taste perversion. H.pylori was eradicated in 32/40 (80%) subjects.

Conclusion: We conclude that triple therapy with omeprazole, clarithromycin and metronidazole for one week is an effective, inexpensive, well-tolerated treatment for H. pylori infection.

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4. RETINOBLASTOMA GENE PRODUCT EXPRESSION IN OESOPHAGEAL CANCER

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Lesions at various genetic loci have been identified in human oesophageal cancer. These include amplification of a number of oncogenes including *c-myc* and *int-2*, loss of heterozygosity (LOH) on chromosome 17p and point mutations within the p53 tumour suppressor gene. Frequent LOH at the retinoblastoma (RB) locus in human oesophageal cancers has been reported. Rb is believed to contribute to carcinogenesis when both chromosome copies are inactivated. Mechanisms of Rb inactivation include point mutation, rearrangement and deletion. However, Rb gene product expression (pRb) has not been examined and the prognostic significance of Rb inactivation has not been addressed.

The aim of the study was to assess Rb gene product expression in oesophageal squamous cell carcinomas and adenocarcinomas and to correlate the findings with survival in patients with these cancers.

Oesophageal cancer samples from 33 patients (14 adenocarcinomas and 18 squamous cell carcinomas) were immunohistochemically assessed for nuclear pRb expression using the mouse monoclonal antibody PMG2.345.

pRb expression was classified as Rb positive or Rb altered depending on the percentage of tumour cells which expressed the protein. Prognostic significance of Rb protein expression was assessed from a Kaplan-Meier and log-rank analysis.

8/14 (57%) and 6/14 (43%) of oesophageal adenocarcinomas were Rb positive and Rb altered tumours respectively. Of 18 squamous cell carcinomas, 12/18 (67%) and 6/18 (33%) were Rb positive and Rb altered tumours respectively.

Conclusion: Our results support the hypothesis that Rb inactivation is involved in the pathogenesis of oesophageal cancer. However, survival analysis studies indicate the Rb protein expression does not bear prognostic significance in these cancers.

5. QUALITY OF LIFE FOLLOWING OESOPHAGECTOMY

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The medical profession tends to have a nihilistic view of the long term survival and functional outcome of oesophagectomy. While the former may be based on factual evidence, too often the latter is based merely on anecdotal misconceptions. In this prospective study we present figures on the level of symptomatology in a group of 129 patients after oesophagectomy. Data from the most recent out-patient visit is presented. Symptoms were graded I to IV, from absent to severe, and values presented are percentages. The median follow up was ten months (range 1 - 192). The most troublesome symptoms were fullness after a meal in 27%; regurgitation on lying in 9%; and heartburn in 10%. 49% were fully active, 39% were ambulant and capable of light work, 9% spent less than 50% of their time in bed, but were capable of total self care. Only 1.7% spent more than 50% of their time in bed and were incapable of self care.

	Absent I	II	III	Severe IV
Anorexia	37.2	29.4	17.1	16.3
Dysphagia	77.5	14.4	6.3	1.8
Vomiting	82.5	15.1	2.4	—
Swallowing	53.1	28.9	13.3	4.7
Fullness	39.7	32.5	22.2	5.6
Heartburn	69.5	19.5	8.6	2.4
Regurgitation	55.5	33.9	8.0	1.6
Odynophagia	97.6	0.8	1.6	—
Resp. Problems	80.7	13.4	3.4	2.5

Conclusion: The majority of patients studied following oesophagectomy had a good symptomatic and functional outcome.

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6. HEPATITIS C THIRD GENERATION SCREENING TESTS - ARE THEY THE ANSWER?

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The diagnosis of HCV infection is initially based on detection of anti-HCV antibodies by enzyme immunoassay supplemented by immunoblot assay. Polymerase chain reaction which confirms viral presence is performed only on sera that have previously tested positive by at least one enzyme immunoassay with immunoblot confirmation. The development of assays for anti-HCV detection has progressed rapidly since 1989 when the viral genome was first described but there had been concerns over the sensitivity and specificity of earlier assays. Third generation enzyme immunoassays and immunoblot assays were introduced in 1993 in an attempt to improve diagnostic accuracy. Extensive data exists on the accuracy of second generation assays but to date no data has been published on the sensitivity and specificity of the new assays.

In this study 1000 serum samples were tested for anti-HCV by two separate third generation anti HCV enzyme immunoassays (Ortho 3 HCV and Murex anti-HCV) and also by third generation immunoblot assay (RIBA3) which is the best serological test currently available and serological status was determined by this result.

The results obtained were as follows

RIBA3	Ortho +	Ortho -	Murex +	Murex -
Positive 351	351	0	341	11
Indeterminate 194	158	36	112	82
Negative 455	178	277	41	414

In summary the Ortho appears to be the more sensitive (100% sensitivity, 60% specificity) of these two enzyme immunoassays but the Murex is more specific (97% sensitivity, 91% specificity). Despite the excellent sensitivity of these assays, our results demonstrate the inadequacies in the specificity of intial serological screening and the requirement for further laboratory investigation.

7. DOES MAINTENANCE THERAPY WITH 5-AMINOSALICYLIC ACID COMPOUNDS SULPHASALAZINE, OLSALAZINE OR MESALAZINE IN PATIENTS WITH INFLAMMATORY BOWEL DISEASE CAUSE RENAL DISEASE

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The nephrotoxic effects of salicylates have been known for many years. Despite widespread use of 5-aminosalicylic acid compounds in the management of inflammatory bowel disease, few cases of renal damage as a result of such therapy have been reported. In this study we investigated this association in patients on maintenance therapy with sulphasalazine (n=20), olsalazine (n=20) or mesalazine (n=20) and in a control population (n=20). Mean duration of therapy for each drug was approximately two years. Analysis was performed on fresh post voiding urine samples, pH adjusted to 6.5 - 7.5, in age, sex and disease matched patients. Renal function was estimated from B2-microglobulin and N-acetyl-D-glucosaminidase (NAG) excretion in the urine, expressed as a ratio of creatinine clearance.

RB2-microglobulin/creatine ratios were 0.033 (0.011-0.084), 0.029 (0.012-0.072), 0.032 (0.0122-0.065) and 0.033 (0.015-0.060) for sulphasalazine, olsalazine, mesalazine and control group respectively. No significant difference was found between the three treatment groups when compared to the control group.

NAG/creatinine ratios were 199.2 (60-504), 190.7 (70-432, 188 (72-380) and 196 (90-360) for sulphasalazine, olsalazine, mesalazine and control group respectively. Once again, no significant difference was observed between the control group and the three treatment groups.

This study demonstrates that there is no statistically significant evidence to suggest that sulphasalazine, olsalazine or mesalazine can cause renal damage in the doses used and for the duration observed.

8. LOCALISED INCREASE IN INTESTINAL γδTCR+ AND CD8+ LYMPHOCYTE POPULATIONS AND ENHANCED CLASS II ANTIGEN EXPRESSION: INDEPENDENT FEATURES OF COELIAC DISEASE

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T lymphocytes are thought to play a significant role in the pathogenesis of coeliac disease. The characteristic lymphocytic infiltrate into both lamina propria and epithelial intestinal compartments can now be subjected to detailed analysis by flow cytometry. The aim of this study was to examine phenotype and class II antigen expression of CD3+ lymphocytes from both the lamina propria and intraepithelial sub-compartments in patients with active coeliac disease.

Small intestinal biopsies were taken from 16 patients, 7 with normal small intestinal histology and 9 with active coeliac disease (all 9 were on gluten-containing diets, had raised levels of gliadin antibodies and abnormal histology). The epithelial layer was removed using chelating and reducing agents; the lamina propria was digested using collagenase. Yields of $0.3\text{-}2.45 \times 10^6$ lymphocytes were obtained from the epithelial layer and $0.17\text{-}1.5 \times 10^6$ from the lamina propria of patients with coeliac disease. These cells were stained with antibodies specific for CD3, CD4, CD8, γδ TCR and HLA-DR molecules. Peripheral blood lymphocytes (PBL) from the same patients were used as controls. Class II antigen expression was detected on a small percentage of PBL. A significant increase was seen in the proportion (mean 38.1%; range 4.98-54.76) of lamina propria lymphocytes from patients with active coeliac disease expressing HLA-DR antigens ($P<0.05$) when compared with controls (mean 20.97%; range 7.46-36.32). Class II antigen expression was similarily increased ($P<0.01$) on intraepithelial lymphocytes (mean 37.85%; range 25.01-53.58) when compared with controls (mean 22.40%; range 3.09-41.05).

T cell phenotypic studies showed an increase (mean 27.95%; range 15.16-51.80) in the CD3+ γδ TCR+ population in the epithelial layer ($P<0.01$) when compared with control patients (mean 10.95%, range 025.70). Surprisingly, the γδ infiltrate into the lamina propria of coeliac patients was much less marked (mean 2.73%; range 0-4.85; controls: mean 0.78%; range 0-1.06). Similarily, a significant change in the CD4:CD8 ratio seen in the coeliac epithelium (ratio 1:22; control epithelium, ratio 1:8) was not mirrored in the lamina propria where the CD4:CD8 ratios remained similar (1.3:1 in coeliac preparations and 1.4:1 in control).

In conclusion, high Class II antigen expression by coeliac intestinal T cells was common to both lamina propria and epithelial layers. The increase in the γδ TCR+ and CD8+ populations, however, was significantly more marked in the epithelial layer than in the lamina propria suggesting that these features may be independent.

9. SERUM VCAM-1 CORRELATES WITH INTESTINAL INFLAMMATION IN PATIENTS WITH INFLAMMATORY BOWEL DISEASE

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VCAM-1 (vascular cell adhesion molecule -1) is expressed on endothelial cells and follicular dendritic cells and is important in mediating adhesion and activation of lymphocytes and monocytes. VCAM-1 may be important in mediating immune responses in inflammatory bowel disease (IBD). We have previously shown that soluble forms of VCAM-1 are elevated in IBD and correlate with markers of disease activity. However, the ideal measure of disease activity marker is the degree of intestinal inflammation as treatment is primarily directed at this endpoint. We therefore measured serum levels of VCAM-1 in patients with

IBD and correlated these with markers of intestinal inflammation.

41 patients with IBD were studied, 33 patients with active disease and 8 patients with inactive disease. Clinical disease activity was measured using the Harvey Bradshaw index. Serum C-reactive protein was measured by an immunoturbometric method and serum VCAM-1 was measured using an ELISA method. Serum VCAM-1 was measured in 14 healthy volunteers. Within 72 hours of serum sampling, colonoscopy was performed and biopsies taken in 23 IBD patients. Intestinal inflammation was quantified using a standard grading system based on a number of parameters including oedema and the degree of leukocyte infiltration.

Serum VCAM-1 was elevated in patients with active compared with inactive IBD; 1839±1284 vs. 429±164 ng/ml, $p<0.01$, and compared to controls, 402±320ng/ml, $p<0.01$. Serum VCAM-1 correlated with the degree of intestinal inflammation; $r=0.54$, $p<0.01$. Serum VCAM-1 also correlated with CRP levels; $r=0.48$, $p<0.05$ but not with the Harvey Bradshaw index; $r=0.15$, p=ns.

Conclusion: Serum VCAM-1 correlates with the grade of intestinal inflammation in IBD patients. Serum VCAM-1 is a useful marker of disease activity in patients with IBD. VCAM-1 may be important in mediating intestinal inflammation in IBD patients.

10. ROUX-EN-Y HEPATICOJEJUNOSTOMY - A DEFINITIVE, SAFE PROCEDURE

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Although Roux-En-Y Hepaticojejunostomy is considered to be the definitive procedure for biliary bypass or reconstruction, the technique is rarely used due to a perception that it is complex and hazardous.

We have used a simple end to side technique with a 60 cm Roux-En-Y limb in 39 procedures on 35 patients. A palliative bypass was performed in 14 cases (unresectable pancreatic carcinoma 10; gall bladder carcinoma 1; cholangiocarcinoma 3) including 9 cases in whom endoscopic stenting was initially attempted in view of the patient's age or extensive disease. A reconstructive procedure was performed in 25 cases (Whipple's operation 6; liver transplant 7; cholangiocarcinoma 2; benign biliary stricture 6; bile duct injury 3; primary sclerosing cholangitis 1).

	PALLIATIVE n = 14	RECONSTRUCTIVE n = 25
Mean age	62.5	48.7
Malignant Disease	13	7
Alive	11	22
Mean Follow-up (months)	5.6	9
Cholangitis	0	3
Post-operative bile leak	0	1
Re-operation	0	3

In conclusion Roux-En-Y hepaticojejunostomy is a safe definitive treatment for benign or malignant biliary tract obstruction. It should be considered in all patients in whom surgery is indicated including those likely to require frequent changing of endoscopic stents.

11. MANOMETRIC FINDINGS IN PROXIMALGASTRO-OESOPHAGEAL REFLUX

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Gastro-oesophageal reflux disease (GORD) is quantified by ambulatory pH studies of the distal oesophagus. A proportion of patients with GORD also reflux into the proximal oesophagus and this is associated with extra-oesophageal symptoms such as asthma, pharyngitis and globus. This study aims to establish whether proximal acid reflux is related to an abnormality in oesophageal motility or clearance of acid refluxate. Manometry and 24 hour dual channel pH monitoring were performed in 105 patients, group 1 (n=42) normal controls, group 2 (n=33) distal GORD, group 3 (n=30) distal and proximal GORD.

RESULTS	Group 1	Group 2	Group 3
pH monitoring			
Proximal oesophagus % time pH <4	0.4 (0.1)	0.2 (0.1)	3 (0.4)*
Distal oesophagus % time pH <4	2 (0.2)	18 (4)	18 (3)
Reflux episodes > 5 min (no.)	1 (0.2)	8 (2)	6 (1)
Manometry (mmHg)			
Proximal wave amplitude	31 (3)	21 (2)	39 (5)*
Middle wave amplitude	39 (3)	32 (4)	47 (6)*
Distal wave amplitude	31 (4)	32 (3)	45 (5)*
Upper sphincter pressure	53 (5)	54 (4)	53 (4)
Lower sphincter pressure	10 (1)	7 (1)**	10 (1)

Mann-Whitney U test values: mean (sem)
*p < 0.01 group 3 v group 1 & 2; **p < 0.01 group 2 v group 1 & 3

Clearance of acid from the distal oesophagus was similar in both reflux groups but peristaltic wave amplitude was higher in the proximal reflux group. Proximal acid reflux cannot be explained by an abnormality of oesophageal acid clearance from the distal oesophagus and is unlikely to be due to an abnormaliity in oeosphageal motility. As pharyngeal swallowing is a further mechanism of acid clearance, pharyngo-oesophageal motility studies may help to elicit the pathogenesis of extra oesophageal manifestations of GORD.

12. A DOUBLE BLIND CROSS-OVER PLACEBO CONTROLLED STUDY OF OMEPRAZOLE IN THE TREATMENT OF THE "SENSITIVE OESOPHAGUS"

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By convention gastrooesophageal reflux disease (GORD) is diagnosed when ambulatory pH monitoring shows distal oesophageal exposure to acid (pH<4) for more than 5% of a 24 hour observation period. Recently the term "sensitive oesophagus" has been proposed to describe patients with less than 5% acid exposure but in whom at least 50% of symptoms coincide with reflux episodes (i.e. significant symptom index (SI)). The aim of this study was to test the validity of the "sensitive oesophagus" by observing the response to acid suppression treatment. Subjects were patients with GORD type symptoms (heartburn and reflux), normal OGD and total oesophageal acid exposure on ambulatory monitoring of less than 5%. 12 patients had a significant SI (ie. "sensitive oesophagus") and 6 non significant. Every patient was given in random order omeprazole 20 mg bd and placebo, each

for 4 weeks, in a double blind, crossover design. Symptoms were assessed by a system of grading and by means of a personal daily diary.

10 of 12 patients (83%) in the "sensitive oesophagus" group reported a better level of overall symptoms with omeprazole compared to placebo ($p<0.02$, Paired Sign test). There was a significant difference in scores for heartburn and reflux (median 2.0 out of a possible 6.0, vs 4.0 ($P<0.01$), average number of days per week with symptoms (4.75 vs 5.8, $p<0.01$), and average number of days per week when additional Gaviscon tablets were used (3.0 vs 5.1 $p<0.01$). Scores for dysmotility type symptoms (fullness, nausea etc.) were not different for the 2 treatments (median 5.5 out of a possible 30, vs 4.25, $p=0.75$). In the group with a non significant SI there were no significant differences for the same comparisons of treatment effects, although one patient clearly benefited from omeprazole.

Conclusion: Omeprazole significantly improves the reflux symptoms of patients with "sensitive oesophagus". A small number of cases do not respond, and a small number who are excluded by the criteria are responsive. We suggest that patients with GORD symptoms, and normal OGD, could reasonably be managed by an adequate initial trial of acid suppression without the need for pH monitoring.

13. BILE ACID TOLERANCE AND DECONJUGATING ACTIVITY OF LACTOBILITY

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The healthy human gastrointestinal tract provides a complex and hostile environment for most microorganisms entering via the oral cavity. These include fluid flow, pH, enzymes, mucous, bile, the mucosal immune system and normal colonising microflora. Little is known about how the microflora have adapted to and survive in this hostile environment. In addition little is known about their metabolic contribution and their contribution to colonisation resistance against gram -ve flora, pathogens, overgrowth, inflammation and the production of cytotoxic products or carcinogens.

We have isolated crypt adhering microflora from freshly resected human small bowel. We have previously reported that these organisms produce potent inhibitory activity against GI pathogens. Here we report on bile tolerance. Of concern is bile salt hydrolysis with the conversion to toxic metabolic products which may be involved in toxicity, inflammation and carcinogenesis.

We have shown that isolated lactobacilli and bifidobacteria are unusually tolerant to high concentrations of bile salts and importantly to human bile at concentrations found in the small intestine. Furthermore we have shown that deconjugation is not an essential feature for bile resistance. We report for the first time a novel inducible bile tolerance mechanism in human GI lactobacilli. Using reverse phase HPLC coupled with pulsed amperometric detection at a gold electrode we have developed a rapid system for the analysis of bile acid metabolic products. We have found that 25% of bile tolerant lactobacilli isolated were capable of deconjugation using a bacterial bile salt hydrolyase. This is significant since all small bowel deconjugation appears to be microbially derived. All bifidobacteria deconjugated bile efficiently. There was a higher substrate preference for glycine conjugates rather than tauline conjugates. Further metabolised bile acid products have also been identified.

In conclusion, we have shown that some human small bowel colonising lactobacilli and bifidobacteria are extremely bile tolerant. Possession of bile salt hydrolase activity is not an essential component for tolerance. Based on these and previously reported findings on bacterial pathogen inhibitor activities appropriate manipulation of the GI flora should have an important role in immune, inflammatory and other metabolic activities in the human GI tract.

14. CYTOKINE PRODUCTION IN PATIENTS WITH *H. PYLORI* INFECTION

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H. pylori infection has been implicated in gastroduodenal inflammation. However, the exact pathogenesis is unclear. The aim of this study was to look for further evidence of immunological mechanism in *H. pylori* associated disease. We measured cytokine IL-2 and IL-4 profiles from peripheral blood lymphocytes (PBL) in 15 dyspeptic patients. Five never experienced *H. pylori* infection demonstrated by negative *H. pylori* IgG antibody. CLO test and histology (Group 1); five experienced *H. pylori* infection but eliminated demonstrated by positive IgG antibody, negative CLO-test and histology (Group 2); five *H. pylori* infected patients demonstrated by positive IgG antibody, CLO-test histology (Group 3). *H. pylori* can stimulate IL-2 and IL-4 production from PBL in *H. pylori* negative as well as positive individuals. The spontaneous IL-2 production was greater in idividiulas who had *H. pylori* IgG antibody alone (Group 2) than that who never experienced *H. pylori* infection (Group 1) or *H. pylori* infected patients (Group 3). IL-4 level in supernatants of cultured PBL was increased in the presence of *H. pylori* in *H. pylori* infected patients (Group 3).

		IL-2 (pg/ml)			IL-4 (pg/ml)		
Groups	No.	Spontaneous	PHA	*H. pylori*	Spontaneous	PHA	*H. pylori*
1	5	17.89 (14.96-21.18)	25.36 (21.18-54.52)	24.61^{+} (22.52-47.24)	18.86 (14.92-22.56)	35.10 (23.30-64.90)	29.88** (26.22-34.38)
2	5	23.29* (17.12-26.86)	31.74 (20.32-49.13)	29.90^{++} (20.75-35.31)	20.15 (18.96-23.24)	46.20 (19.92-54.12)	29.88** (26.22-36.48)
3	5	18.28 (14.96-21.18)	39.13 (24.39-74.44)	26.86^{++} (21.18-41.83)	19.31 (17.48-20.24)	36.10 (35.0-60.65)	35.0** $^{+}$ (32.20-43.40)

$^{*}p<0.05$ vs group 1 & 3 in IL-2 or vs group 2 in IL-4 respectively; $^{*}p<0.01$ vs group 1; $^{+}$ or $^{++}$ $p<0.05$ or <0.025 vs spontaneous and **or $^{\wedge}$ $p<0.005$ or <0.001 vs spontaneous respectively.

In conclusion, increased type 2 cytokine production by *H. pylori* may play an important role in immune pathogenesis of *H. pylori* infestation.

15. BONE REMODELLING INDICES AND SECONDARY HYPERPARATHYROIDISM IN COELIAC DISEASE

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Aims: To determine the prevalence of secondary hyperparathyroidism and to assess bone remodelling indices in a group of Irish coeliacs.

We studied 27 patients with coeliac disease: *Group 1* newly diagnosed coeliacs (n=14); *Group 2* coeliacs who have responded clinically and histologically to a gluten-free diet (n=8); Group 3 patients unresponsive to a gluten-free diet and immunosuppressive therapy [Refractory Sprue] (n=5).

Patients had serum drawn for intact parathyroid hormone (PTH), 25-hydroxy vitamin D [25(0H)D], ionized calcium, alkaline phosphatase and biochemical markers of bone formation: procollagen I carboxyterminal propeptide (PICP) and osteocalcin (Oc). In 18 patients urinary indices of bone resorption - pyridinium crosslinks and hydroxyproline (OHP) - were measured in a two hour urinary collection. Vitamin D status was considered deficient at serum 25(0H)D < 25nmol/l, borderline at < 50 nmol/l and adequate when > 50 nmol/l.

The prevalence of secondary hyperparathyroidism (HPT) was 21%, 12.5%, and 40% in groups 1, 2 and 3 respectively. Prevalence rates for vitamin D deficiency in each of the groups were the same as those for HPT. Borderline vitamin D levels of < 50nmol/l were found in 57% of group 1, 25% of group 2 and 100% of group 3. Bone formation indices fell within the reference range for all three groups: mean PICP ± 1 standard error (SE) were 162.9 ± 19.1, 143.6 ± 19.7 and 112.9 ± 14.6 in groups 1, 2 and 3 respectively. Mean Oc ± 1 SE for group 1 was 22.0 ± 5.65, 11.6 ± 0.88 for group 2 and 19.7 ± 2.95 for group 3.

Bone resorption markers were slightly greater than the normal mean in group 1 but most elevated in group 3: mean urinary pyridinoline ± 1 SE was 38.3 ± 8.8 for group 1, 29.2 ± 2.3 for group 2 and 59.1 ± 24.7 for group 3. Mean urinary deoxypyridinoline ± 1 SE were 9.13 ± 1.83, 6.93 ± 0.85 and 11.04 ± 4.3 in groups 1, 2 and 3 respectively.

Hypovitaminosis D and secondary hyperparathyroidism are common in newly diagnosed coeliacs. These changes are less frequent in those who respond to a gluten-free diet. Patients with refractory sprue have a high prevalence of secondary hyperparathyroidism. Bone formation is normal in all groups. A remodelling imbalance is apparent, as increased bone resorption is evident in both new and refractory groups.

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16. RANITIDINE AND OMEPRAZOLE AND THEIR COMBINATION WITH ANTIBIOTICS IN THE ERADICATION OF HELICOBACTER PYLORI

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Eradication of Helicobacter pylori prevents relapse of duodenal ulceration. However the optimum regime for H pylori eradication remains unclear. Omeprazole and ranitidine and their combination with antibiotics have been shown to be effective. The former has been studied extensively but not the latter. In addition, few studies have compared the two drugs directly.

Aim: To compare omeprazole plus antibiotics versus ranitidine plus antibiotics in the eradication of H pylori.

80 patients (mean age 48 years, range 18-75) who had H pylori infection as assessed by endoscopy and histology, culture and 13C-urea breath test (UBT) were randomised in a double-blind manner to either a 2 week regime of omeprazole 20 mg daily, amoxycillin 500 mg tid and metronidazole 400 mg tid (OAM) or omeprazole 20 mg daily and clarithromycin 500 mg tid (OC) or omeprazole 20 mg daily and placebo (OP) or ranitidine 600 mg bd, amoxycillin 500 mg tid and metronidazole 400 mg tid (RAM). Repeat endoscopy for histology and culture and 13C-UBT were undertaken 4 weeks after completion of therapy. Eradication of H pylori was successful if all three tests were negative.

H pylori was eradicated in 6 of 19 patients in the OAM group (32%); 4 of 15 in the OC group (27%); none of 18 in the OP group (0%); 8 of 18 in the RAM group (44%). Eradication rates were significantly higher in the OAM, OC and RAM groups compared to OP ($p<0.05$) but were not significantly different to each other. Overall metronidazole resistance was 58% and was similar in all groups. Eradication rates in metronidazole sensitive patients were 71% and 100% for OAM and RAM respectively. Compliance ranged from 90-96% for the groups. Side effects occurred in 2/20 (10%), 1 of whom withdrew in the OAM group; 4/20 (20%), all of whom withdrew in the OC group; 2/20 (10%), 1 of whom withdrew in the OP group; 2/20 (10%), both of whom withdrew in the RAM group.

H pylori eradication rates using high dose ranitidine plus amoxycillin and metronidazole is similar to that of omeprazole in combination with the same antibiotics or clarithromycin. Eradication rates were low due to a high incidence of metronidazole resistance. The eradication rate was higher in metronidazole sensitive patients. There was a high incidence of side effects in the omeprazole and clarithromycin group.

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17. THE USE OF CYCLOSPORIN IN THE MANAGEMENT OF ACUTE EXACERBATIONS OF INFLAMMATORY BOWEL DISEASES.

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In recent years the use of cyclosporin in the management of inflammatory bowel disease has been investigated. Reports of the benefit of such therapy however are conflicting. In this study we examined the short term outcome of cyclosporin treatment in severe Crohn's disease (n=14) and Ulcerative Colitis (n=7). In each case the patients' disease was unresponsive to high dose parenteral steroids. Duration of disease ranged from 4-21 years for Crohn's disease and 1-5 years for Ulcerative Colitis. All patients were on concomitant therapy with a 5-aminosalicylic acid compound and the steroid dose was not changed during the course of cyclosporin therapy. Cyclosporin was administered at infusion rate of 4mg/Kg/24 hours for five days then orally at 200mg bd. Duration of therapy ranged from 2 months to 6 months.

Treatment outcome was monitered by patient self-assessment and physicians assessment of disease severity and the avoidance of surgery in those for whom it seemed inevitable. Biochemical, haematological and renal responses were monitered by daily estimation of albumin, white cell count, erythrocyte sedimentation and creatinine.

All but one of the patients with Crohn's disease improved both subjectively and clinically to therapy and all avoided surgical intervention. In four of the seven patients with Ulcerative Colitis however surgery proved to be unavoidable. Two patients recovered from the acute episode and were asymptomatic on oral cyclosporin therapy at review.

This study demonstrates the effectiveness of cyclosporin therapy in acute exacerbations of Crohn's disease refractory to steroid therapy but questions the use of such therapy in the management of patients with fulminant Ulcerative Colitis.

18. FLOW CYTOMETRIC ASSESSMENT OF BONE MARROW MICROMETASTASES IN PATIENTS WITH GASTROINTESTINAL MALIGNANCIES. A PROSPECTIVE EVALUATION - INITIAL REPORT

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Although the prognostic significance of lymph node micrometastases in patients with colorectal cancer has been questioned, there is evidence in support of bone marrow micrometastases as a prognostic marker. Since the true significance of micrometastases may relate to the actual number of micrometastatic cells, flow cytometry may be preferable to immunohistochemical assessments. We have previously reported the use of flow cytometry as a reliable and accurate means of identifying and quantifying micrometastatic deposits of neoplastic epithelial cells within bone marrow. This was validated in a controlled 'spike' experiment in which varying numbers of neoplastic epithelial cells were added to bone marrow samples and flow cytometry was performed in a blinded fashion. We now report our results with the first 100 patients studied consecutively by flow cytometry. These consisted of 48 patients with colorectal cancer, 12 with oesophageal, 24 with gastric and the remainder had non-gastrointestinal tract cancers. Bone marrow was taken at the time of surgery in a manner that avoided any contamination by skin epithelia. Epithelial cells were detected using fluorescence labelled monoclonal antibodies to cytokeratins. Bone marrow micrometastases were found in 25% of patients with colo-rectal cancer, 33% of those with oesophageal, 21% of those with gastric and 36% of those with other types of (non-gastrointestinal) cancer. The majority of patients with colorectal cancer who had micrometastatic bone marrow deposits were staged conventionally as Dukes B or C. One patient with a Dukes A cancer was found to be positive for micrometastases, but a repeat bone marow sample at postoperative follow-up tested negative. Only a minority of colorectal cancer patients with micrometastases had unequivocally elevated levels of CEA (13%) and CA 19-9 (25%). In conclusion, flow cytometric assessment of bone marrow can detect micrometastatic deposits at an early stage in 20-30% of patients with gastrointestinal malignancies. Prospective evaluation of these patients which is continuing will establish the significance of the findings in terms of a potential influence on survival.

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19 EARLY EXPERIENCE WITH LAPAROSCOPIC NISSEN FUNDOPLICATION

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The introduction of laparoscopic procedures has dominated the surgical literature in recent years. Laparoscopic cholecystectomy is firmly established but the situation with appendicectomy, herniorrhaphy and colectomy is far from clear. Laparoscopic anti-reflux surgery has only been recently described. We audit our initial experience with laparoscopic Nissen fundoplication (LNF).

Since 1993, 21 patients have undergone LNF in this unit. There were 10 males and 11 females of average age 46 years (range 24-70 years). The mean duration of symptoms was 48 months and all had biopsy proven oesophagitis secondary to reflux, and unresponsive to treatment with Omeprazole. Symptomatology included retrosternal pain (n=15), epigastric pain (n=10), reflux (n=6), nausea and vomiting (n=3) and belching (n=2). All patients had pre-operative pH studies with a mean total time pH < 4 of 17.5% (range 6.8-38.3).

Seventeen patients had successful completion of LNF, four patients were converted to open operations because of technical problems. Mean hospital stay for those successfully undergoing LNF (n=17) was 4.9 days and there were no complications in this group. Twelve patients are well on follow-up, 3 had dysphagia, one of whom remains well after dilatation, and 3 patients have recurrent reflux.

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20. LAPAROSCOPIC GASTRIC FUNDOPLICATION: AN ADVANCE IN THE SURGICAL MANAGEMENT OF GASTRO-OESOPHAGEAL REFLUX DISEASE

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Gastro-oesophageal reflux (GOR) is often refractory to medical therapy. Nissen fundoplication at laparotomy remains the standard surgical treatment, but is associated with significant morbidity. Many patients are considered unsuitable for open surgery. Laparoscopic gastric fundoplication holds appeal for the reduction of surgical trauma and post-operative morbidity. We report a consecutive series of patients with refractory GOR managed by laparoscopic Nissen fundoplication.

In a one year period, 12 patients underwent laparoscopic fundoplication. Six were male and 6 female. Median age at operation was 42.5 years (range: 23-68 yrs). Symptoms (heartburn, regurgitation, dysphagia) were graded pre-operatively using the DeMeester scoring system. All patients underwent upper GI endoscopy. 24-hour pH monitoring and oesophageal manometry were performed in 11 patients.

All operations were performed by one surgeon (OJM). Laparoscopic access was via 5 ports (10 mm x 4; 5 mm x 1). Dissection was performed using an ultrasconic scalpel. The hiatal slings were dissected to create a posterior oesophageal window. The mediastinum was entered, preserving the vagi. Two or 3 short gastric vessels were clipped before creating a 1 to 2 cm floppy wrap by drawing the posterior stomach behind and to the right of the oesophagus using shaped memory grasping forceps. The wrap was secured and the hiatus closed using 2/0 silk. Mean operating time was 115 minutes (range 60-210 min.). There was one conversion to an open procedure following camera failure. There were no other intra-operative complications.

Median post-operative stay was 3 days (range: 2-6 days). All patients reported a dramatic improvement in symptomatology following surgery. Clinical improvement was graded using the DeMeester system and the Visick scale and was statistically significant. For this reason, post-operative endoscopy, pH monitoring or manometry have not been performed routinely.

Two patients required re-admission within 2 weeks of surgery (mean follow-up: 184 days; range: 46-361 days). Both had subjective symptoms of dysphagia and underwent upper GI endoscopy, which was normal. In both cases symptoms settled within 2 to 3 days and both remain well on follow-up at 9 and 3 months. No patient developed the gas-bloat syndrome.

Laparoscopic gastric (Nissen) fundoplication allows surgical correction of refractory GOR with minimal trauma to the patient. The laparoscopic procedure is as effective as the traditional open

approach and is associated with minimal post-operative morbidity. This approach allows early mobilisation, discharge from hospital and return to normal activity. The advent of laparoscopic gastric fundoplication has significant implications for the surgical management of gastro-oesophageal reflux disease.

21. BERBERINE INHIBITION OF ELECTROGENIC CHLORIDE SECRETION IN HUMAN DISTAL COLON IN VITRO

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We have previously shown berberine, an alkaloid which has anti-diarrhoeal actions, to inhibit cAMP dependent chloride secretion in rat distal colon. We have now extended this study to human tissue. In these experiments we have examined the effects of the alkaloid on electrogenic chloride secretion, cAMP generation and cAMP dependent protein kinase (PKA) activation in human distal colonic mucosae. Chloride secretion in voltage clamped human colonic mucosae in response to carbachol (1-500μM; EC_{50} = 4.7±1.2μM; max Δ Short Circuit Current = 95.6±21.4μA/cm^2) was significantly inhibited by berberine (100μM; n=5; $p<0.05$). In separate experiments, mucosal levels of cAMP (50.8±35.4 fmol/μg protein; determined by radioimmunoassay) were increased by forskolin (10μM; 227.9+91.5 fmol/μg protein; $p<0.05$). This effect was not inhibited in the presence of 100μM berberine (268.4±61.4 fmol/μg protein) or 500μM berberine (221.4±79.5 fmol/μg protein). Using an enzymatic assay which determines phosphate incorporation from donor AT32P into kemptide by PKA, basal levels of PKA activity in human distal colonic segments were 3.4±0.7ngATP/μg protein. These levels were elevated in tissues stimulated with dibutyryl cAMP (500μM; 6.2±1.4 ngATP/μg protein; $p<0.05$). PKA activation was not altered by 100μM berberine (4.8±0.8 ngATP/μg protein) or 500μM berberine (5.6±0.2 ngATP/μg protein; n=5 throughout). In summary, we have shown that berberine inhibits carbachol stimulated electrogenic ion transport in human distal colon (which is due at least in part to cAMP dependent mechanisms). However the alkaloid did not influence cAMP generation or cAMP dependent protein kinase activity indicating that an alternative mechanism must account for the inhibitory action of berberine on colonic chloride secretion.

22. PHARYNGO-OESOPHAGEAL CLEARANCE MECHANISMS IN GASTRO-OESOPHAGEAL REFLUX DISEASE

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Primary and secondary oesophageal peristalsis constitute the main clearance mechanisms of gastro-oeophageal reflux. The contribution of each is unclear and prolonged physiological studies of pharyngeal activity in relation to reflux have not been performed. The aim of this study was to evaluate pharyngeal and oesophageal body responses to reflux episodes in a population of patients with gastro-oesophageal reflux disease. Eight patients with GORD were studied. Ambulatory manometry of the pharynx and oesophagus in addition to 24 hour pH monitoring was performed. Pharyngo-oesophageal manometric responses to reflux in the upright and supine state were compared. The manometric response to reflux was defined as motor activity occurring during the reflux episode and continuing until clearance was complete.

	Upright	Supine
Mean duration of reflux episode (secs)	42	135*
Mean delay to Pharyngeal Contraction (secs)	15	75*
% reflux episodes cleared by Single Transmitted Swallow	34	97*
% reflux episode associated with		
Transmitted swallow	70	94*
Non Transmitted Swallow	31	4*
Secondary Oesophageal Peristalsis	63	5*

*$p<0.05$ Wilcoxon Rank Sum

In the supine state the predominant mechanism of clearance was by transmitted swallow. There were few non-transmitted swallows or secondary oesophageal body contractions. Although there was a significant delay in initiation of the swallow, reflux was almost invariably cleared by a single transmitted swallow. This is in contrast to upright reflux in the same patient population where an average of three swallows was required. The pharyngo-oesophageal response to reflux is significantly different during sleep and explains the longer duration of reflux episodes while supine.

23. CELLULAR REGULATION OF POTASSIUM RECYCLING IN HUMAN COLON

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The osmotic gradient for water absorption in human colon is provided by transcellular sodium ion (Na+) absorption. Potassium ion (K+) recycling at the serosal membrane of the human colon epithelial cells is essential in order to maintain a favorable electrical gradient for sodium ion (Na+) absorption. Fluid secretion into the colon lumen is driven by chloride (Cl-) secretion. To maintain cellular electrical homeostasis during Cl-secretion, K+ is extruded at the serosal membrane of the cell. Therefore K+ channels in the serosal membrane of the epithelial cells of human colon play a pivotal role in overall fluid transfer. In this study we documented the mechanisms of the cellular regulation of K+ recycling.

Isolated epithelium from normal human colon was mounted in temperature controlled Ussing chambers, and the spontaneous transmembrane voltage was clamped to 0 millivolts by a short circuit current (SCC). SCC quantifies electrogenic ion transfer. The luminal membrane of the epithelial cells, was rendered electrically neutral by permeabilising it with nystatin thereby allowing the serosal membrane to be studied in isolation.

Greater than 95% of the K+ dependant SCC was inhibited by tolbutamide. The remaining K+ current was inhibited by tetra-pentyl-ammonium (TPA). The tolbutamide sensitive K+ channels were inhibited by increase in cellular protons (H+), free calcium (Ca++), or adenosine-tri-phosphate (ATP). The TPA sensitive channels were stimulated by increase in cellular Ca++, and by cellular pH < 7.5.

Stimulation of protein kinase C (PKC) with phorbol ester causes a pH dependant increase in K+ recycling by the tolbutamide sensitive K+ current. This latter effect was inhibited by clamping intracellular

pH or by inhibition of the Na+/H+ exchanger with amiloride (100μM), or by increasing cellular cyclicadenosine-mono-phosphate (cAMP) using forskolin (50μM).

The tolbutamide sensitive K+ channel has previously been demonstrated to play a vital role in Na+ absorption by the human colon epithelium. The TPA sensitive K+ channel is responsible for maintainance of cellular homeostasis during Ca++ induced Cl- secretion. Because these latter processes determine overall fluid transfer, a proper understanding of the cellular regulation of K+, recycling is essential if fluid transfer across the human colon is to manipulated.

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24. EXPANDABLE METAL ENDOPROSTHESIS AND RADIOTHERAPY IN THE PALLIATION OF MALIGNANT DYSPHAGIA: AN EIGHTEEN MONTH FOLLOW UP

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Optimal palliation of malignant dysphagia is not yet established. Our early encouraging experience involving an expandable metal endoprosthesis in combination with palliative radiotherapy has continued, allowing assessment of efficacy, complications and medium term follow up.

Thirty two patients (30-86 yrs, male : female 18 : 14) with inoperable oesophageal cancer had a mean dysphagia score of 2.6 (Mellor and Pinkas). Regarding tumour location, 20 were distal third/gastro oesophageal junction, 9 mid and 3 proximal oesophagus. The mesh stents were inserted under fluoroscopic guidance requiring only intra venous sedation in 3 of 32. This was followed within three weeks by palliative dose external beam radiotherapy in 26 patients.

Successful placement was achieved in 30/32 (> 90%). The only serious early complication was a single misplacement, with removal and perforation. The mean dysphagia score one week post insertion was 0.7. During a mean follow up of eight months (range 1-18), the mean dysphagia score remained < 1.0. The thirty day mortality was 16%. 28 of 32 patients were discharged home. Only 6 patients (19%) were readmitted (five for less than one week duration), for management of dysphagia with 8 episodes of tumour ingrowth/overgrowth, treated with bougiage, ethanol injection or laser therapy. There were 4 episodes of bolus impaction cleared easily endoscopically. The mean survival at most recent follow up is 20 weeks (range 1-18 months).

The combination of expandable metal endoprosthesis with radiotherapy provides rapid, sustained and good quality palliation of malignant dysphagia, with a low rehospitalisation rate and survival comparable to other palliative modalities.

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25. SPECTRUM OF OESOPHAGEAL SENSITIVITY IN PATIENTS WITH GASTRO-OESOPHAGEAL REFLUX DISEASE

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A wide range of symptom severity occurs in gastro-oesophageal reflux disease (GORD) and is known to correlate poorly with the presence or degree of oesophagitis or measures of oesophageal acid exposure, some patients experiencing troublesome heartburn from apparently minor degrees of reflux, whilst others with erosive oesophagitis may present only with complications such as anaemia. Considerable evidence now links the occurrence of gastrointestinal symptoms in patients with functional gastrointestinal disorders to alterations in visceral sensory function, with lowered thresholds for the perception of visceral discomfort being recorded in irritable bowel syndrome, functional dyspepsia, and non-cardiac chest pain. We hypothesised that differing degrees of visceral sentience might explain the range of severity of symptoms in GORD. We therefore measured the sensory thresholds for perception and discomfort evoked by balloon distention of the oesophagus above the level of any mucosal change in four different groups of subjects: healthy volunteer controls (n = 15); patients (n = 11) with heartburn (4 with oesophagitis) found at pH monitoring to have excess oesophageal acid exposure (> 6.9% of a 23 hour study) and a positive symptom index (≥50%) for reflux events; patients (n = 20) with heartburn (none with oesophagitis) found at pH study to have acid exposure within the physiological range but a positive symptom index for reflux events; and patients with Barrett's oesophagus (n = 9). Patients with excess symptomatic reflux exhibited similar sensory thresholds (mean ml [±sem]) to healthy controls for both perception (11.3 [±2.2] vs. 12.1 [±1.5] (n.s.)) and discomfort (17.3 [±2.1] vs. 16.4 [±1.4] (n.s.)). Patients with symptomatic but not excess reflux however demonstrated significantly lower thresholds than controls; perception 7.7 [±0.9] ($p = 0.002$) and discomfort 10.4 [±0.8] ($p < 0.0001$) whilst patients with Barrett's oesophagus, on the other hand had significantly higher sensory thresholds than controls; perception 17.2 [±1.9] ($p = 0.002$) and discomfort 20.4 [±1.6] ($p = 0.02$). The results indicate a spectrum of visceral sensitivity in GORD with patients who apparently perceive physiological reflux symptomatically at one end and those with Barrett's oesophagus at the other. Altered visceral nociception may be an important aspect of more than just functional gastrointestinal disorders.

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26. ASSESSMENT OF TOTAL BODY POTASSIUM IN ILEOSTOMY PATIENTS BY BIO-ELECTRICAL IMPEDANCE ANALYSIS

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Ileostomy patients are known to suffer from depletion of total body potassium (TBK) but only 2% of TBK is extracellular, and serum K^+ levels may be normal in the presence of intracellular K^+ depletion. Bio-electrical Impedance Analysis (BIA) is a new technique used to estimate human body composition and nutritional status. This method measures resistance and reactance to an alternating current passed through the body. Resistance (R) is primarily due to cell membrane resistance, whereas reactance (Xc) is due to the capacitance of body tissues.

This study assessed the role of BIA as a rapid and non-invasive bedside method of assessing total body potassium in healthy ileostomy patients.

10 normal volunteers and 9 ileostomy patients participated in the study. All had fasted for a minimum of 6 hours before the tests. Measurements were taken using the Akern (BIA) 101/S and a K^+40 whole body counter. In addition, the ileostomy patients had serum and urinary K^+ levels measured on the day of testing. The data was analysed using multiple linear regression.

TBK was significantly reduced in ileostomy patients compared with controls: 145.9 ± 12.2 grams v. 109.3 ± 5.8 grams

(P=0.018, t-test). In normal volunteers, BIA allowed very accurate prediction of TBK using the equation derived from linear regression: $K^+ = 1.24\ Xc + (0.00416)H^2 - 1.59\ W - 0.538\ R$; (r=0.99, p=0.003). Height (H) and weight (W) alone were unable to accurately predict TBK: (r=0.5, p=ns). When the equation derived from healthy controls was applied to the ileostomy group, there was a significant correlation between predicted and actual TBK: (r=0.71, p=0.03).

BIA is an accurate predictor of total body potassium in normal healthy subjects and may be useful in predicting total body potassium in ileostomy patients.

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27. IgM ANTIBODY TO HEPATITIS C - A POSITIVE CORRELATE WITH PCR

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Conventional serology for Hepatitis C Virus by enzymeimmunoasay (ELISA) provides evidence of infection, past or present, but does not indicate infectivity. Recombinant immunoblot assay (RIBA) correlates rather better with the presence of HCV genome by the polymerase chain reaction (PCR) which currently tends to be the "gold-standard" for infectivity. However, both the latter are slow, subjective to a degree, labour-intensive and therefore expensive. In many viral infections the appearance of IgM antibodies provides an earlier indication of acute infection and persistent low levels often suggest chronicity. We have evaluated a new test for IgM anti-HCV in comparison with PCR. In 42 patients infected previously with HCV, we compared established "inhouse" PCR and Roche Amplicor PCR with a recently introduced IgM anti-HCV test (Abbott Diagnostics, Weisbaden, Germany). 29 (69%) were positive for both IgM anti-HCV and PCR and 5 were negative for both. In 7 cases PCR was positive but IgM anti-HCV was not found and in one case IgM was positive and PCR was negative but the patient had been treated with interferon. Thus there was a 100% positive correlation between the presence of IgM anti-HCV and the presence of HCV genome in untreated patients ($\chi 2 = 1104$, $p<0.001$), but not necessarily the reverse. We conclude that IgM anti-HCV testing (at approximately one-third of the cost of PCR) may provide an economical aid to minimising the need for a significant proportion of PCR testing for HCV genome.

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28. A VALIDATED SYMPTOM SCORE FOR DYSPEPSIA IN CLINICAL TRIALS

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Most clinical trials of patients with dyspepsia use symptoms as an outcome measure. However, there is currently no validated method of measuring symptom severity in dyspeptic subjects. Three attributes are necessary for use of symptom scoring systems as outcome measures in clinical trials; reproducibility, responsiveness to change and validity. We have designed a questionnaire for recording frequency and severity of dyspeptic symptoms, scored on a sliding 5-point Likert scale with a maximum of 38 and a minimum of 4. We have now assessed this questionnaire with regard to reproducibility, responsiveness and validity.

1. Reproducibility: One author interviewed 64 subjects (33 male), including 30 healthy volunteers and 34 dyspeptic patients (T0). The interview was repeated one week later, prior to diagnostic or therapeutic intervention (T1).

The mean score of the normal subjects at T0 was 6.9 and at T1 was 6.56.

The mean score of the dyspeptic subjects at T0 was 18.18 and at T1 was 17.12. There was no significant difference between T0 and T1 in both populations.

2. Responsiveness to change: Dyspepsia scores were compared before (T0) and four weeks after (T2) eradication of H. pylori in non-ulcer dyspepsia (NUD) patients and acid reduction in reflux oesophagitis (GORD) patients. The mean T0 and T2 symptom scores in the NUD group were 16.25 (range 6-27) and 8.25 (range 3-19) respectively ($p<0.01$) and in the GORD group were 18.57 (range 8-27) and 15.85 (range 8-24).

3. Validity: This was assessed by comparing the dyspepsia scores in the healthy volunteers and patients with upper G.I. diseases. The mean score in the normal population was 6.9 (range 4-12) and was significantly lower than that in the dyspepsia subjects 18.18 (range 6-34).

This questionnaire for assessing the severity of dyspepsia is reproducible and has high responsiveness and validity. It will be a valuable tool for assessing the response to treatment in patients with dyspepsia.

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29. SERUM GST AS AN EARLY INDICATOR OF DISEASE RELAPSE FOLLOWING TREATMENT WITHDRAWAL IN AUTOIMMUNE CAH

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Previous studies have shown that conventional biochemical, immunological and histological indices are of limited value in predicting outcome of steroid withdrawal in patients with autommune chronic active hepatitis [CAH]. Hepatic glutathione-S-transferases [GST] form a complex group of enzymes that provide an alternative to measurement of aminotransferase activity for detection of hepatocellular damage and has been shown to be superior to the conventional biochemical indices in the setting of paracetamol-induced hepatic damage, hepatotoxicity following anaesthetic agents and acute liver allograft rejection.

Aim: to prospectively evaluate the use of serum αGST as an early indicator of disease reactivation in a group of patients with CAH in whom treatment was being withdrawn over a ten week period.

Nine consecutive patients [8F:1M] with CAH in disease remission, defined by serum AST < 40IU/L on three consecutive monthly determinations, were studied. Dosage of corticosteroids and/or azathioprine was reduced every two weeks until therapy was discontinued or relapse, defined as a serum AST > 120 IU/L, occured. Disease activity was monitored fortnightly by clinical examination, liver function tests [LFTs] and serum αGST [Hepkit, Biotrin International].

Five patients [55.5%] experienced relapse of disease as defined by increase in AST > 120IU/L. In all 5 patients, serum αGST levels increased from baseline normal values [<8ng/ml] 2-4 weeks prior to elevation in standard aminotransaminases [AST/ALT] with a mean value of 36.5 ± SD 43.1ng/ml, median 22.2 [range 10.9-112.8]. Following reintroduction of therapy,

aGST levels fell to within normal range before a subsequent similar fall was observed in AST. Serum αGST remained within the normal range in patients in whom treatment was successfully withdrawn.

The results indicate that serum αGST is a sensitive indicator of disease reactivation in patients with autoimmune CAH.

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30. LACK OF CLINICAL AND IMMUNOLOGICAL EVIDENCE OF AUTOIMMUNE DISEASE IN ANTI D ASSOCIATED HEPATITIS C VIRUS INFECTION TYPE 1B

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Previous studies have suggested that Hepatitis C Virus (HCV) infection is associated with a high prevalence of autoimmune disease. High titres of autoantibodies have been detected in HCV infection. We have investigated patients chronically infected with HCV due to anti-D immunoglobulin with respect to symptoms and signs of autoimmune disease and presence of autoantibodies/cryoglobulins.

48 female patients, mean age: 43.1 years (range 25 - 55 years) infected with HCV contaminated anti-D Immunoglobulin in 1977 (41), 1979 (2),1990 (2), 1991 (3) were surveyed clinically and by full autoimmune serology to ANA, AMA, LKM-1, SM, Thyroid microsomal, Thyroid globulin, parietal cell antibodies and cryoglobulins. There were no patients with specific symptoms or signs suggestive of autoimmune disease. Two patients complained of generalised musculoskeletal symptoms but without demonstrable physical signs. Cryoglobulins were not detected in any patient. In only 6 patients (12%) were thyroid microsomal antibodies detected (and in 2 of these, thyroid globulin antibodies were also positive). These patients were all clinically euthyroid but 2 had borderline normal thyroid function tests. In 3 patients (6%) ANA titres were weakly positive and in 5 patients (10%) gastric parietal antibodies were positive. In particular no antibodies to LKM-1 were noted.

This unexpected finding of lack of autoimmune phenomena in chronic Hepatitis C may be explained by the preponderance in our study group of HCV serotype 1B, by the long period of infection and/or by the low HCV innoculum at infection.

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31. OATS IS NOT IMMUNOGENIC IN COELIAC DISEASE

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The pathogenesis of coeliac disease is considered to involve an immunological response to specific wheat proteins (gliadins) and similar fractions in the closely related cereals barley and rye. The issue of oats toxicity is controversial and was investigated in this study. Oats challenge was performed in 8 coeliac patients in clinical and histological remission. Each patient ingested 50g of oats on a daily basis for 3 months and clinical, laboratory and immunological markers of disease activation were measured. To-date 7 patients have completed the study. All patients remained asymptomatic throughout the challenge and none demonstrated laboratory evidence of malabsorption. No mucosal damage was evident on routine histological evaluation; a small increase in intraepithelial lymphocytes was noted in 2 patients. Furthermore, oats did not cause immunological activation, since no increase in endomysial antibodies, gliadin antibodies or MHC class II staining of enterocytes was found. This study demonstrates that oats is not toxic in coeliac disease and oats appears not to contain the immunogenic peptide found in gliadin.

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32. INDUCTION OF A HEAT SHOCK RESPONSE PROTECTS TUMOUR CELLS FROM MONOCYTE MEDIATED LYSIS

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Tumour cells proliferate despite the presence of tumouricidal mediators. We hypothesise that this could be due to the induction of a heat shock response in the tumour cell. The heat shock response appears to represent a universal cellular defense mechanism in native host cells. Tumour cells may also utilise this mechanism to protect themselves from host defense mechanisms by increasing either intracellular levels or surface expression of heat shock proteins (HSP). The aim of this study was to assess the effect of heat shock induction on tumour cell protection against host effector cells. The heat shock response was induced by either sodium arsenite (SA, 0-320μM for 6hrs) or by hyperthermia (42°C for 20mins). For the cytotoxicity assays the colorectal tumour cell line, SW707, were heat shocked, labelled with ^{51}Cr and incubated with human monocytes (M0) isolated from healthy volunteers. Cytotoxicity was assessed by ^{51}Cr release. Flow cytometric studies on heat shocked SW707 cells to assess surface expression of HSP60 and HSP70 were by an indirect method.

	% cytotoxicity SW707 heat shocked	HSP60 expression (MCF)	HSP70 expression (MCF)
control	40.45±0.812	10.17±0.150	17.13±0.664
40μM SA	0.40±0.403*£	10.29±0.086	13.81+1.769
80μM SA	2.63±1.756*£	10.53±0.196	13.77+2.103
160μM SA	3.18±1.719*£	9.86±0.239	13.72+2.236
320μM SA	0.77±0.765*£	10.28±0.230	12.45±2.112
42°C	11.50±0.799*	11.05±0.346	14.72+2.687

n=mean±SE *p<0.002 Vs control £p<0.001 Vs 420C Student's t-test.

The data indicate that heat shocking tumour cells significantly protects them from MO mediated tumour cell lysis. Since the flow cytometric data indicate that there is no significant increase in surface expression of HSP60 and HSP70 on the tumour cell when the cells are heat shocked, it is reasonable to infer that the induction of intracellular HSP levels are responsible for the protective effect on the tumour cells.

SA: sodium arsenite Mø: monocyte HSP:heat shock protein MCF:median channel fluorescence.

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33. DOES OESOPHAGEAL MOTILITY AS MEASURED BY SOLID BOLUS OESOPHAGEAL SCINTIGRAPHY IMPROVE AFTER HEALING OF EROSIVE OESOPHAGITIS?

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Reflux oesophagitis is often associated with oesophageal dysmotility, and opinion is divided as to whether the dysmotility

is a primary disorder or secondary to reflux damage to the oesophageal wall. Cranford et al (Br. J. Surg. 1985; 72(12): A1037) have previously suggested that oesophageal scintigraphy (OS) using the solid egg bolus technique they described is a useful screening test for motility disorders.

Aims/methods: To assess oesophageal motility using solid bolus OS in reflux oesophagitis patients and determine the effect of healing on motility. Patients with grades II-III esophagitis (modified Savary-Miller) underwent OS before and after treatment with omeprazole 20mg BD for 8-13 weeks. OS was performed by asking patients to chew and then swallow a 10ml bolus of poached egg white radiolabelled with Tc-99m, while standing in front of a gamma camera. Patients then took a dry swallow every 20 sec for 4 minutes while the camera took serial 1 sec images. After a drink to clear the oesophagus, the study was then repeated.

Twenty patients (13 male), mean age 43 yrs (range 23-63), with erosive oesophagitis (15 grade II, 5 grade III) underwent OS, the results of which were analysed by time-activity curves and condensed image analysis whereby swallows are classified as normal, oscillatory, step delay, non-specific delay or non-clearance.

Eleven patients (55%) had abnormal OS before treatment. Two patients failed to heal after 13 weeks treatment (healing rate 90%), both had grade II oesophagitis and normal OS initially. Seventeen of the 18 healed patients had repeat OS: 10 of 11 remained abnormal, 5 of 6 remained normal, 1 improved from abnormal to normal, and 1 changed from normal to abnormal (probably representing a patient whose initial study failed to detect abnormal motility). Ten of the 12 patients who had abnormal OS either before or after treatment showed either a step-delay (7) or oscillatory (3) transit pattern and the remaining 2 patients had a non-specific abnormality. The duration of oesophageal transit (time-activity curve) did not correlate with grade of oesophagitis or symptom severity.

OS using the solid egg bolus technique is a useful screening test for motility disorders in patients with reflux oesophagitis, showing an abnormality in 12 (60%) patients in this series. In those patients with abnormal oesophageal transit demonstrated by OS, healing of oesophagitis does not improve the abnormal motility, suggesting that the abnormal motility is a primary phenomenon.

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34. BRADYKININ STIMULATES HUMAN COLONIC ION TRANSPORT IN VITRO

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It has previously been demonstrated that bradykinin (BK) stimulates ion transport across intestinal epithelia via mechanisms that involve eicosanoids and enteric neurons. Although BK stimulates ion secretion in human intestinal epithelial cell lines, there is little known of its effects in intact human tissue. In this study we have examined the effects of BK on ion tranport across human colon. Segments of human colon, stripped of their underlying smooth muscle, were voltage clamped by the continous application of short circuit current (SCC). BK added to the basolateral domain of the tissue stimulated a concentration dependent inward SCC with an EC_{50} of 0.6±0.2μM (n=17). Responses to BK were mimicked by the B2 receptor agonist, lys-BK, but not by the B_1 agonist, des arg^9-BK. The loop diuretic, bumetanide (100μM; n=5) significantly attenuated responses to BK, indicating that these responses are due, at least in part, to electrogenic chloride secretion.

Responses to BK were reduced by the cyclooxygenase inhibitor, piroxicam, the lipoxygenase inhibitor, nordihydroguairetic acid (NDGA) and the neurotoxin, tetrodotoxin (TTX).

Treatment	% reduction of control
Piroxicam (10μM)	48±10.3*
NDGA (100μM)	70.8±18.3*
TTX (1μM)	58.7±5.7*

(*p<0.05 compared to control)

In separate experiments BK added to the apical domain of the tissue stimulated a more rapid onset and transient response than basolateral addition with an EC_{50} of 4.7±1.0nM (n=5). Responses to apical BK were also mimicked by B_2 but not B_1 receptor agonists.

We conclude that BK stimulates ion transport in human colon by the activation of B2 receptors. These responses are due, at least in part, to electrogenic chloride secretion. *In vivo* this may contribute to secretory diarrohea associated with intestinal inflammation.The full expression of secretory responses to BK involves eicosanoid production and ampilification by the enteric nervous system. In future experiments we aim to more fully elucidate these mechanisms. The mechanisms by which apical bradykinin stimulates ion transport in this tissue remain to be identified.

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35. INGESTION OF E.COLI INDUCES PMN APOPTOSIS THROUGH AN OXYGEN-DEPENDENT MECHANISM

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Apoptosis (Apop) is a distinct mechanism by which eukaryotic cells die. Neutrophils (PMNs) play a fundamental role in the systemic inflammatory response syndrome. The removal of PMNs from the normal and septic host occurs by apop but the induction of this process is unknown. The aims of this study were to determine whether the reactive oxygen intermediates (ROI) induced during the ingestion (Ing) of E.coli would induce apop in human PMNs. PMNs were isolated from 10 healthy volunteers. $1x10^6$ PMN/ml were cultured with different ratios of PMN:E.coli (1:0, 1:5, 1:10, 1:25), for 12 hours. Apop was then assessed by Prodidium Iodide DNA staining, morphology, gel electrophoresis and FcRIII receptor expression. There was a significant increase in ingestion of E.coli and ROI release in response to E.coli after 2 hours of coculture. This correlated with a significant (p<0.05) induction of PMN apop after 12 hours on incubation with E.coli at a ratio of 1:15 and 1:25 PMN:E.coli as well as a significant decrease in FcRm.

PMN:E.coli	1:0	1:5	1:10	1:25
% Ing	0±0	29±8*	34±9*	50±6*
ROI	207±46	257±31	381±71*	782±165*
FcRIII	1210±44	1210±44	430±21*	177±76*
% Apop	23±5	29±2	36±3*	67±6*

Data = Mean ± SD. Stats = ANOVA with p<0.05.

To further investigate the role of ROI in the inducation of PMN apop co-culture experiments with the antioxidants DMSO, Glutathione (GSH) and N-acetycysteine (NAC) were performed. There was a significant (p<O.O5) decrease in E.coli-induced PMN apoptosis on incubation with DMSO 1.0%, GSH 25 mM and NAC 25mM with % apop been 51±2, 48±6 and 35±7 respectively compared to the PMN:E.coli (1:25) alone inducing 67±6% PMN apop. This study demonstrates that E.coli ingestion induces PMN apop through a oxygen-dependent mechanism. The re-

moval of effete PMNs by the process of apop rather than necrosis should be protective and may represent a beneficial mechanism for the host.

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36. PRO-INFLAMMATORY CYTOKINE IL-1 PRODUCTION BY HUMAN INTESTINAL EPITHELIAL

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A primary role in intestinal epithelial lymphocyte activity has been hypothesised for intestinal enterocytes because of their high expression of Class II antigens and their intimate association with CD4+ as well as CD8+ T lymphocytes. For effective initiation and mediation of T lymphocyte function, however, local production of regulatory cytokines such as IL-1 would be required. The aim of this study was to determine if human enterocytes expressed IL-1 message and protein using reverse transcriptase polymerase chain reaction (RT-PCR) and flow cytometry.

mRNA was prepared from the epithelial layer preparations of human small intestinal tissue obtained at surgery and endoscopy; cDNA was synthesised using reverse transcriptase. IL-1 mRNA was detected using PCR and IL-1 specific primers. IL-1 mRNA was also detected in cDNA preparations from the human intestinal cell line, I407.

Specific mRNA is not always translated; it was important therefore to determine whether intestinal epithelial cells produced IL-1 protein as well as mRNA. To this end, we developed a method for detecting cytoplasmic cytokine in specific cell populations using flow cytometry. Cells were permeabilised using a detergent so that internal antigens could be stained with specific antibodies while maintaining cellular architecture and surface marker expression. Epithelial cell preparations from four patients in whom small bowel disease was excluded were studied. In all patients, the epithelial cells, which stained positive with the epithelial cell specific antibody, BER-ep 4, also had cytoplasmic IL-1. A small sub-population of CD3+ intraepithelial lymphocytes had detectable Il-1; the rest were negative for cytokine specific staining.

Evidence of IL-1 mRNA and protein in human small intestinal epithelial cells supports the hypothesis that the normal entrocyte is involved in entrocyte-intraepithelial lymphocyte communication. This interaction may aid gastrointestinal immune surveilance.