Inflammatory bowel disease T59–T70

W57

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

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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

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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.

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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

T60

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

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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.

T61

SOCIAL CLASS, ETHNICITY & SMOKING IN A NATIONALLY REPRESENTATIVE COHORT WITH CROHN'S DISEASE.

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Introduction: There are few studies concerning the frequency of Crohn's disease (CD) among different ethnic groups within the UK. Likewise, there are few reports concerning the relevance or not of social class. Smoking has been shown to be a risk factor for CD. The fourth Morbidity Statistics in General Practice (MSGP-4) represents a study of why patients in England or Wales visit their GP. It was conducted in 1991-92 and covered a population of 468,042. Data concerning ethnicity, social class, economic activity, smoking status (in the last week) and living in an urban or rural environment had been collected in MSGP-4.

Methods: We have previously identified 291 patients reported to have CD and in whom the diagnosis was not refuted subsequently. Controls were selected from the same practice as the case and matched for gender and age. Data were analysed in conditional logistic regression models using the GLIM4 program. Odds ratios (OR) for individual factors were obtained with 95% confidence intervals.

Results: 1682 controls were selected. The OR for afro-caribbean's was 3.19 (95% confidence interval 0.28-36.53) and for Indians 1.03 (0.10-10.98). Compared with social classes 1&2 the OR for social class 3-non-manual was 1.21 (0.79-1.85), 3-manual 0.70 (0.48-1.03) and 4&5 1.08 (0.72-1.64). There was no significantly increased risk of being unemployed, OR 0.63 (0.29-1.36) but there was an increased risk of being registered as permanently sick, OR 4.01 (2.21-7.29). Those with Crohn's disease were more likely to smoke, OR 1.29 (0.95-1.75) and less likely to live in a rural environment, OR 0.75 (0.44-1.28).

Conclusion: This confirms that CD is more likely to occur in those who smoke and live in an urban environment. Social class is not a significant factor for CD. It may be more common in those of afro-caribbean but not Indian origin.

T62

THE ASSESSMENT OF QUALITY OF LIFE IN INFLAMMATORY BOWEL DISEASE PATIENTS: A FIVE ITEM SCALE FOR OUTPATIENT USE.

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Quality of life (QOL) has been identified as an important measure in the evaluation of patient response to treatment in IBD patients. QOL or well-being has also been shown to be a strong determinant of clinical course (e.g., outpatient visits, subjective pain or symptom complaints & patient satisfaction). Previous studies by our group have suggested that QOL in IBD is complex and is influenced by disease activity and psychosocial factors. The purpose of this study was to develop a brief easily administered self-report scale to assess QOL in an outpatient clinic. Recent data by our group indicates that the IBDQ, a disease-specific measure of QOL can be predicted by five questions from the entire 32 item questionnaire (Gastroenterol 1995; 108: A931). We enrolled 133 consecutive patients with IBD (94 Crohn's, 29 U.C.) who completed the IBDQ and the five item questionnaire (IBDQ-SF). Disease activity was recorded in 69 Crohn's (Dutch AI and CDAI) and 18 UC patients.

Results: There was a strong correlation between the IBDQ-SF and the total score of the IBDQ ($r=0.91, p<0.001$). The IBDQ-SF score also correlated with the 4 subscales of the IBDQ (ie) Bowel symptoms, $r=0.79$ $p<0.001$; Emotional functioning, $r=0.88$ $p<0.001$; Systemic symptoms, $r=0.88$ $p<0.001$; Social functioning, $r=0.77$ $p<0.001$. For the Crohn's patients the CDAI was strongly related to QOL (ie, $r=-0.71$, $p<0.001$ with the IBDQ Total, with significant correlations [$p<0.001$] with the four subscales as well), whereas there was no relationship between the Dutch AI and QOL (ie, $r=-0.27, p>0.05$ for the IBDQ Total).

Conclusion: The five item questionnaire, the IBDQ-SF is a quick and reliable means to measure QOL in an IBD Clinic setting. This scale will allow clinicians to identify patients whose disease-specific QOL is impaired and who may need specialized care addressing psychosocial issues which will facilitate clinical care. The CDAI also correlates with measured QOL in Crohn's disease whereas the Dutch AI, a more objective measure of disease activity does not.

T63

LOW APPENDICECTOMY RATES IN PATIENTS WITH ULCERATIVE COLITIS: EFFECT IS INDEPENDENT OF SMOKING.

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It has recently been noted that there is an inverse relationship between appendicetomy and ulcerative colitis (UC) in a Belgian study. This has not yet been confirmed in the U.K. There is also a possibility that this relationship may be linked with smoking. This case-controlled, prospective study examines the frequency of appendicetomy and smoking in a stable U.K. population.

Methods: A total of 228 patients with a definitive diagnosis of inflammatory bowel disease were prospectively interviewed by a standardised questionnaire. There were 112 patients with a diagnosis of ulcerative colitis and 116 patients with Crohn's disease. 112 controls were recruited from accident and emergency department and were matched for age and sex.

Results: The appendicectomy rates amongst patients with UC was 2.7% (3/112) which was significantly lower compared to 16.1% (18/112) amongst controls ($P<0.005$, Odds ratio: 6.96, 95% C.I. 37.7-1.93). 2 patients with UC had their appendicetomy performed after diagnosis. Appendicectomy rates amongst patients with Crohn's disease was significantly greater than controls: 34% (18/112) vs. 16.1% (39/116), ($P<0.01$; O.R. 2.65, 95% C.I. 5.31-1.34) but includes 9 patients who had appendicectomy as part of surgical intervention for Crohn's disease. The strong association of UC with non-smoking is confirmed (2% v 42%, $P<0.001$, OR 35.6; 95% C.I. 308-8.68). However there appears to be no association between smoking and appendicetomy in all three groups.

Conclusion: UC is associated with significantly lower appendicetomy rates than controls while appendicetomy rates in Crohn's disease is higher. This finding is independent of smoking. This intriguing finding may have implications in the aetiology of inflammatory bowel disease.

T64

THE ARTICULAR DISTRIBUTION AND CLINICAL COURSE OF THE PERIPHERAL ARTHROPATHIES IN ULCERATIVE COLITIS (UC).

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The association between UC and peripheral arthropathy is well recognised, but there are no diagnostic criteria and no information on their long term outcome. We have reviewed the records of patients with UC currently under review in Oxford and by subdividing them into 'reactive'(ReA), peripheral(PeA) and arthralgia have assessed the frequency and clinical course of the arthropathies.

The case notes were reviewed for the presence of arthritic symptoms and signs. Reactive arthropathies involved less than 5 joints including a weightbearing joint with recorded evidence of joint swelling or effusion. Peripheral arthropathies involved 5 or more joints with evidence of swelling or effusion. Poorly defined joint symptoms and complaints without joint swelling were classified as arthralgia. Coincident rheumatic complaints, general features of UC and other extraintestinal manifestations were also recorded.

There were 860 patients under active review, of which 117 (13.6%) complained of peripheral joint problems. 43 (5.0%) had arthralgia, 27 (3.3%)ReA and 22 (2.6%) PeA. In addition 6 patients had known rheumatoid arthritis and 39 had osteoarthritis. 23 of 27 patients (85.2%) with ReA had an episodic course, of which 7 (30.4%) had more than one episode, and 4 (14.8%) had a continuous course. The most commonly affected joint was the knee, being affected in 23/27 cases with an effusion present in 10. Of 22 patients with peripheral arthritis 18 (82.8%) had a continuous course from 10-180 months, 2 (9.1%) were episodic, and 2 (9.1%) had only a single attack. The metacarpophalangeal joints were the most commonly involved (14/18) and there were no joint effusions recorded.

We have demonstrated two distinct forms of peripheral arthropathy in UC with different distributions and natural histories; ReA, which occurs as a single episode in 59.3% of cases and pursues a continuous course in only 14.8%, and PeA which pursues a continuous course in 82.8% of cases and only occurs as a single episode in 9.1%.

T65

BONE DENSITY AND BONE TURNOVER MARKERS IN PATIENTS WITH CROHN'S DISEASE. R.J.Robinson, S.J.Iqbal, R.P.Whitaker, F. Al-Azzawi, K.Abrams, J.F.Mayberry. Gastrointestinal Research Unit, Leicester General Hospital, Gwendolen Road, Leicester.

Patients with Crohn's disease (CD) are at risk of osteoporosis but the underlying pathophysiology is unclear. The aim of this cross-sectional study was to assess bone mineral density (BMD) in CD and use bone turnover markers to investigate the possible mechanisms of bone loss.

Methods: 100 patients with Crohn's disease (52 premenopausal females, 48 males, mean age 38.7 (13.2)) had BMD measured at the hip and lumbar spine by dual energy x-ray absorptiometry (Osteoporosis: BMD > 2.5 SD below young adult, osteopenia: BMD >1 - < 2.5 SD below young adult). Bone formation markers measured were osteocalcin (Bone gla protein, BGP), bone specific alkaline phosphatase (BALP) and pro-collagen carboxy-terminal propeptide (PICP). Urinary deoxypyridinoline (dPyr) was measured to assess bone resorption. Results are presented as mean (SD)(normal range). Total alkaline phosphatase, serum calcium and phosphate were measured on all patients and 25(OH) D measured on patients with low bone density. Two patients had biochemical osteomalacia and were excluded from the analysis.

Results: 61 (62%) patients had reduced BMD (Osteoporosis=7, osteopenia=54). Mean BGP was 9.0 (3.3)(normal range 3.4-10 ng/ml); BGP levels were increased in 34 (35%) of the 98 patients but none had values below the normal range. Mean BALP was 17.5 (13.5) (normal range 11.6-43.3 U/L); 2 (2%) patients had increased BALP and in 21 (21%) BALP was lower than normal. Mean PICP was 98.1 (47.6) (normal range 69-163 ng/ml); PICP was high in 7 (7%) patients with 24 (24%) patients recording values below the normal range. Mean dPyr was 10.2 (6.7) (normal range 2.5-6.5 nMDpd/mM Creat.); 72 (73%) of the 98 patients studied had elevated dPyr. Bone formation markers were not significantly different in patients with low bone density but dPyr was significantly reduced (9.1 (5.4) vs 12.0 (8.2), p=0.04). Urinary dPyr was not significantly associated with any formation markers but was positively correlated with BMD at Ward's triangle (r=0.23, p=0.02). PICP was negatively correlated with BMD at the lumbar spine (r= -0.24, p=0.02) and trochanter (r= -0.22, p=0.03). Bone turnover markers were not significantly different in the 23 patients taking corticosteroids.

Conclusion: This study confirms that low BMD occurs commonly in patients with CD, and a high proportion of patients have increased bone resorption as evidenced by raised dPyr. Our results suggest that a variety of mechanisms may operate to produce low BMD in patients with CD.

T66

NON-STEROIDAL ANTI-INFLAMMATORY DRUGS (NSAID'S) SALICYLATES AND COLITIS - A STRONG ASSOCIATION Gleeson M H Hardman J V, Clinton C, Spencer D. The General Hospital, Jersey, Channel Islands, UK.

In a prospective study of 62 consecutive new cases of colitis from November 1993 to May 1996, a careful drug history revealed 45 patients who had been taking NSAID's or salicylates. Mean age of patients with NSAID-associated colitis was 40.5 years (range 20 - 77) and of others (idiopathic colitis) was 49.9 (range 23 - 90).

No patients had a previous history of colitis, faecal cultures were negative and diagnosis was always established by colonoscopy. Patients with NSAID-associated colitis had either; (1) distal disease identical in endoscopic appearances to ulcerative colitis (59%) or (2) proximal disease with rectal sparing and discontinuous disease, similar to Crohn's colitis.

Histology revealed crypt abscesses, ulceration and a mixed inflammatory infiltrate but no granulomatas. All patients received standard medical treatment for inflammatory bowel disease and 18 (40.0%) of the NSAID group required systemic steroids compared to 8 (47.1%) of the idiopathic group. One patient in the NSAID group required procto-colectomy. 15 patients with NSAID-associated colitis have relapsed (the majority being in the distal colitis group). Relapses have occurred without further exposure to NSAID's or salicylates, and this group of patients appear to be developing a chronic colitis.

It could be argued that these observations were a coincidence due to the high level of exposure of the general population to NSAID's and salicylates. We therefore carried out a control study. A drug history was obtained from 513 attenders at a minor injuries clinic. The age range of this series was 18 - 90 (mean 38) years. 38 (7%) were taking NSAID's or salicylates. This compares to 45 (72.4%) of 62 patients taking NSAID's or salicylates in the colitis group. This gives an odds ratio of 33.1 (95% confidence interval), significantly supporting a causal association between these agents and colitis. They appear to be an important aetiological trigger factor in inflammatory bowel disease.

T67

BALSALAZIDE IS MORE EFFECTIVE AND BETTER TOLERATED THAN MESALAZINE IN ACUTE ULCERATIVE COLITIS (UC).

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This study compared the tolerability and efficacy of balsalazide (mesalazine prodrug) and mesalazine (pH-dependent delayed release) in acute UC.

Patients (101 total, 99 evaluable) (62 male) aged 41 ±13 years (mean ±SD) with grade 2 (erythema+loss of vascular pattern+contact bleeding: 55% of patients), 3 (+spontaneous bleeding: 32% of patients) or 4 (+frank ulceration: 13% of patients) (extent>12cm; left-sided disease 80%) sigmoidoscopically verified, symptomatic (moderate 69% or severe 31%) UC were randomised, double blind, to receive balsalazide 2.25g t.d.s. (equivalent to 0.78g mesalazine) (n=50) or mesalazine 0.8g t.d.s. (n=49) for 4, 8 or 12 weeks, as necessary. Rectal hydrocortisone p.r.n. was provided as relief medication. Both groups were comparable at entry.

A greater proportion of patients achieved symptomatic remission (none/mild symptoms) at 2 (64% vs 43%, p<0.05), 4 (70% vs 51%, p>0.05), 8 (78% vs 45%, p<0.001) and 12 weeks (88% vs 57%, p<0.001) after balsalazide treatment compared to mesalazine. Similarly, more patients achieved complete remission (none/mild symptoms, sigmoidoscopy grade 0 (normal) or 1 (erythema with loss of vascular pattern) with no steroid use in previous 4 days) in the balsalazide group after 4 (38% vs 12%, p<0.01), 8 (54% vs 22%, p<0.005) and 12 weeks (62% vs 37%, p<0.05) with greater patient satisfaction (12 weeks: 91% vs 63%, p<0.005). Diary card analysis showed that patients taking balsalazide experienced more days with complete symptom relief (using no steroid) during the first 4 weeks of treatment (24% vs 14%, p<0.01) and took less time to achieve their first completely symptom free day (median: 10 vs 25 days, p<0.005) compared to those taking mesalazine. One patient in each group discontinued due to an unacceptable adverse event (AE) however, all 4 serious AEs (complications of UC) occurred in the mesalazine group and fewer patients in the balsalazide group reported AEs (48% vs 71%, p<0.05). Analysis of prognostic factors identified low ulcerative colitis grade at entry (p<0.05) and treatment with balsalazide (p<0.01) as increasing the probability of complete remission after 12 weeks.

In conclusion, balsalazide 2.25g t.d.s. was more effective than mesalazine 0.8g t.d.s. in achieving both symptomatic and sigmoidoscopic remission of acute ulcerative colitis and was associated with a better adverse event profile.

T68

IMPRESSIVE HISTOLOGIC IMPROVEMENT AFTER TNF ANTIBODY (cA2) THERAPY IN ACTIVE CROHN'S DISEASE. Baert F, Peeters M, D'Haens G, Geboes K*, Ectors N*, Rutgeerts P. Dpt of Gastroenterology and Pathology* University of Leuven, Belgium.

TNF-α is an important mediator of inflammation in Crohn's disease (CD). We studied the histologic effects of cA2 therapy in a systematic way. Biopsies of 13 patients with active CD before and 4 weeks after a single placebo or cA2 infusion (5, 10 or 20 mg/kg) were reviewed by one blinded pathologist. Six biopsies were taken per patient at every occasion in the most inflamed areas. If ulcers were present, biopsies were taken in the vicinity. Seven pts had colitis, four ileocolitis and two ileitis only. Assessment of the severity of inflammation was based on epithelial alterations, inflammatory changes, the presence of ulcerations and/or granulomas and the number of biopsies affected. A score from 0-3 was given for each item according to severity. The minimal score was 0, the maximum 16.
In addition to classical H&E, immunohistochemical stainings for HLA-Dr, CD68 (activated macrophages), ICAM-1 and LFA-1 were performed using an indirect immunoperoxydase method.

Results: The mean total activity score in the cA2 treated group dropped from 6.7 (2-12) to 3.0 (0-7) in ileitis and from 7.6 (2-12) to 3.0 (0-8) in colitis compared to 11 (10-12) before and 9 (6-12) after in placebo pts. The changes in the inflammatory components of the score were most pronounced. The enhanced epithelial HLA-Dr and endothelial ICAM-1 and numbers of CD68+ monocytes and LFA-1 + lymphocytes observed at week 0 markedly decreased in CD pts treated with cA2 but not in the placebo group.

Conclusion: Monoclonal TNF-α antibody therapy significantly improves histologic disease activity in active Crohn's ileitis and colitis. The improvement is mainly due to a dramatic decrease of the inflammatory infiltrate parallel to a reduction of HLA-Dr expression and the number of CD68+ and LFA1+ cells.

Liver T71–T85

T69

GUT LUMINAL NEUTROPHIL MIGRATION DEPENDS ON THE ANATOMICAL SITE OF CROHN'S DISEASE

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Background: Gut luminal neutropils can be studied by using whole gut lavage fluid (WGLF) obtained after bowel cleansing by a polyethylene glycol-electrolyte solution (Klean-Prep, Norgine). Cytology or an enzyme-substrate reaction to detect granulocyte elastase (GE), can be performed on unfiltered WGLF. The chemoattractants responsible for luminal neutrophil migration are unknown, but bacterial products are potential candidates.

Methods: *70* patients with well characterised active CD underwent whole gut lavage. The clear fluid obtained after complete gut cleansing was stored at -70° C. GE was assayed using the specific chromogenic substrate L-Pyroglutamyl-L-prolyl-L-valine-p-nitroanilide (Quadratech, Epsom). The lower limit of detection by this assay was 39 nkat/L. Patients were divided in to 6 groups according to the distribution of macroscopic disease.

Results: 21 out of 31 patients with Crohn's colitis had detectable GE, (median 238, range <39-2742 nkat/L), whereas only 1 out of 10 patients with small bowel involvement had detectable GE (median <39, range <39-215 nkat/L; P<0.005 Vs colonic disease). 10 out of 13 patients with both small bowel and colonic disease had detectable GE (median 180, range <39-1709 nkat/L; p<0.02 Vs small bowel). In the 6 patients with recurrent small bowel disease who had prior ileocaecal resection, 5 had detectable GE, and the concentrations were significantly higher (median 228, range <39-1240nkat/L) than in those with small bowel disease but no resections (p <0.05). 6 patients with an ileostomy and 3 patients with perianal disease alone had undetectable GE. A patient with Crohn's colitis had received long-term tetracycline therapy for possible tropical sprue before his diagnosis was established - he had no detectable GE in lavage fluid. A high GE concentration of 848 nkat/L was detected in a patient with small bowel CD, who had received high dose long-term NSAIDs for ankylosing spondylitis.

Conclusion: Neutrophil migration into the lumen of the gut is a feature of colonic but not small bowel Crohn's disease suggesting that bacterial flora-derived neutrophil chemoattractants may play a role in gut neutrophil migration. This hypothesis is further supported by the high GE concentrations found in recurrent small bowel disease after ileocaecal resection.

T71

GRAFT INFILTRATING LYMPHOCYTES IN LONG TERM LIVER TRANSPLANTS. A MARKER OF TOLERANCE?

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Clinical tolerance is present in a significant proportion of long term liver transplant recipients, although to date it has not been possible to reliably identify these recipients by conventional clinical, or biochemical, parameters. The present study examined whether immunohistochemical analysis of pre-weaning liver biopsies could discriminate unique patterns in patients subsequently found to be tolerant.

Methods: 37 biopsies: 27 from patients with stable graft function 5 years, or more post transplant, and 10 from patients experiencing acute rejection were studied. Anti-CD3, CD8, CD4, CD45RO, CD45RA, CD56, and CD68 staining was performed.

Results: Of the 27 long term patients, 20 subsequently underwent weaning of their immunosuppression; 6 were tolerant, 5 achieved substantial reductions in their immunosupression (partially tolerant), and 9 were unable to achieve any substantial reduction (intolerant). Reduced numbers of lobular $CD8^+$, and $CD3^+$ cells were present in the tolerant as compared to the intolerant grafts (median 15 vs 22 $p<0.01$, 15 vs 26 cells/high powered field [hpf] respectively, $p<0.03$). Similarly, there were fewer numbers of lobular $CD3^+$, $CD8^+$, $CD45RO^+$ cells in tolerant grafts when compared with acute rejection (16 vs 32 $p<0.01$, 15 vs 31 $p<0.01$, 17 vs 24 $p=0.03$ median cells/hpf respectively). Unexpectedly more lobular $CD45RO^+$ were present in tolerant when compared with partially tolerant patients (17 vs 10 mean cells/hpf, $p<0.05$).

Conclusions: In all long term liver grafts, infiltrating lymphocytes are present, and with those grafts from patients intolerant of immunosuppression withdrawal they are phenotypically similar to those during acute cellular rejection. Fewer CD8 positive cells correlate with graft tolerance, and may aid in the identification of patients in whom successful withdrawal of immunosupression may be undertaken.

T70

TWO-STAGE GENOME-WIDE SEARCH IN INFLAMMATORY BOWEL DISEASE: STRONG EVIDENCE FOR SUSCEPTIBILITY LOCI ON CHROMOSOMES 3, 7 AND 12

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Concordance rates in siblings and twin pairs have provided strong evidence that genetic predisposition is important in the pathogenesis of the inflammatory bowel diseases (IBD). However, neither Crohn's disease (CD) nor ulcerative colitis (UC) has a simple Mendelian pattern of inheritance; the model which seems most pertinent to inflammatory bowel disease is that of a heterogeneous group of polygenic disorders. In the present study, a systematic two-stage search of the human genome for susceptibility genes in inflammatory bowel disease was performed, involving 186 affected sibling pairs.

In the first stage, 89 sibling pairs with IBD were genotyped at 260 microsatellite markers spanning the 22 autosomes, using fluorescence labelled primers for polymerase chain reaction, and semi-automated DNA fragment sizing technology. Allele sharing in affected sibling pairs provided evidence for linkage with 12 markers in 5 distinct regions of chromosomes 2, 3, 7, 12 and 15 ($p<0.001$ for an individual marker, or adjacent markers each with $p<0.01$).

In the second stage, a further 97 affected sibling pairs with IBD were genotyped; linkage for the clustered markers on chromosomes 3, 7 and 12 was confirmed.

Combining data from the first and second stages provided striking evidence for linkage with 3 adjacent markers on chromosome 12-D12S83 ($p = 2.66 \times 10^{-7}$, lod score 5.47), D12S92 ($p = 2.14 \times 10^{-6}$, lod score 4.59) and D12S368 ($p = 5.03 \times 10^{-5}$, lod score 3.29) - and three adjacent markers on chromosome 7 - D7S519 ($p = 1.33 \times 10^{-4}$, lod score 2.89), D7S669 ($p = 8.20 \times 10^{-5}$, lod score 3.08) and D7S524 ($p = 8.45 \times 10^{-4}$, lod score 2.14).

Individual markers on chromosome 2 (D2S142) and chromosome 16 were linked with susceptibility to UC ($p = 0.0071$) and CD ($p = 0.009$), respectively.

These data provide compelling evidence for the chromosomal location of IBD susceptibility genes. Strong candidate genes are present in each of the regions which have been implicated, and are currently being studied.

T72

MONITORING OF LIVER OXYGENATION BY NEAR INFRA-RED SPECTROSCOPY.

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Adequate oxygenation of the liver is fundamental to the outcome of liver surgery and orthotopic liver transplantation(OLT). Near Infra-Red Spectroscopy (NIRS) is a novel technique which can non invasively measure tissue oxy(HbO_2), deoxy(Deoxy Hb) and total haemoglobin(THb). The results of NIRS on the liver have not previously been compared with blood flow.

Large Landrace pigs (n=7) underwent laparotomy under general anaesthesia. Electromagnetic flowmeters were placed around the portal vein and hepatic artery and the NIRS probes on the surface of the right lobe. NIRS and flow were recorded continuously. Baseline readings were taken on achieving a steady state and subsequent recordings following occlusion of the hepatic artery (HA), portal vein (PV) and both (HA and PV).

The correlation between NIRS and hepatic blood flow is shown.

Parameters	HA clamp	PV clamp	HA & PV clamp
HbO_2 (umol/l)*†	-27 ± 23	-165 ± 104	-226 ± 92
Deoxy Hb (umol/l)*†	-6 ± 26	-36 ± 48	-54 ± 40
HA (ml/min)†	0	178 ± 128	0
PV (ml/min)†	817 ± 191	0	0

*changes relative to baseline. † values are in mean±SD.

The baseline HA and PV blood flow were 148±110 and 741±170 ml/min. THb showed a strong correlation with total hepatic blood flow (r=0.932, p<0.001).

We would conclude from this study that NIRS would be an accurate non invasive method of assessing organ perfusion and oxygenation in patients undergoing liver resection or transplantation.