

# PostScript

## LETTERS

### Toll-like receptor 4 gene in IBD: further evidence for genetic heterogeneity in Europe

There is now strong evidence implicating the enteric flora in the aetiopathogenesis of inflammatory bowel disease (IBD), and identification of CARD15 (NOD2) as a pattern recognition receptor (PRR) has given novel insights into host-bacteria interactions. CARD15 is implicated as the intracellular sensor of muramyl dipeptide, a highly conserved bacterial peptidoglycan motif, and raises the question of whether other PRRs are involved in the pathogenesis of Crohn's disease (CD).

Toll-like receptor 4 (TLR4), in combination with CD14, LBP, and MD-2, acts as the PRR for the lipid A moiety of lipopolysaccharide, a major component of gram negative bacteria. Two common cosegregating polymorphisms of this gene have been described in humans, Asp299Gly and Thr399Ile. Asp299Gly has been associated with reduced bronchial responsiveness following lipopolysaccharide stimulation<sup>1</sup> although recent data have questioned the functional effect of this variant.<sup>2</sup>

We therefore note with interest the article of Franchimont and colleagues reporting the Asp299Gly frequency in a Belgian population with IBD (*Gut* 2004;53:987–92). Variant alleles were associated with both CD and ulcerative colitis (UC) in two cohorts and the allele was preferentially transmitted from carriers to affected subjects in a transmission disequilibrium test.

However, apparently contradictory data from elsewhere in Europe highlight the difficulties of interpretation of genetic association studies from single populations. Torok *et al* examined the presence of both the Asp299Gly and Thr399Ile polymorphisms in a smaller German IBD population.<sup>3</sup> In contrast with the Belgian data, this group identified an association of Thr399Ile with UC but there was no association with Asp299Gly, and no association with CD. In addition, we have previously published data on Asp299Gly in 480 Scottish patients with IBD and found no association with either CD or UC.<sup>4</sup>

Why are these data sets discrepant? Issues relating to statistical power, population stratification in case control studies, and phenotypic heterogeneity within IBD may

contribute but we suggest more detailed examination of these data (table 1) provides evidence for genetic heterogeneity between populations in Europe.

CD genotype frequencies were very similar in the Leuven and Edinburgh data sets, and not significantly different from the German CD results. However, allelic frequencies in healthy controls were significantly different in the Scottish population compared with European controls (8.8% *v* 4.6%; *p* = 0.008, odds ratio 1.47 (confidence interval 1.2–3.5)).

It is clearly relevant that a strong suggestion of heterogeneity between populations was given in the original description of Arbour and Lorenz<sup>5</sup> where control population allelic frequencies for Asp299Gly ranged from 3.3% to 7.9% in French and North American data sets. It is noteworthy that these allelic variants were absent in the Japanese population.<sup>5</sup>

Moreover, there is now compelling evidence for genetic heterogeneity between populations for CARD15 in both healthy controls and CD patients. Variant alleles are absent from Asian CD and control populations and exist at a lower frequency in African American CD patients and Ghanaian controls (carriage 1%).<sup>6,7</sup> Carriage frequencies in CD patients approaching 50% have been documented in Caucasian populations from North American and Central Europe. There is also striking evidence for heterogeneity within Europe, and evidence for a geographical North-South gradient in gene effect, as for the CFTR delta 508 mutation in cystic fibrosis. Lower CARD15 (NOD2) frequencies in CD have been reported from Scotland<sup>4</sup> and Finland<sup>8</sup> and are absent in a small Icelandic population.<sup>9</sup>

These data illustrate further the real difficulties in candidate gene analysis in complex diseases. It is likely that the contribution of individual genetic determinants will differ between populations. We suggest that further genetic (as well as functional) data are required before the exact contribution of inherited variants of the TLR4 gene can be confirmed.

**I D R Arnott, G-T Ho, E R Nimmo, J Satsangi**  
Western General Hospital, Edinburgh, Scotland, UK

Correspondence to: Dr I D R Arnott, Western General Hospital, Crewe Rd, Edinburgh EH4 2XU, Scotland, UK; [ian.arnott@doctors.net.uk](mailto:ian.arnott@doctors.net.uk)

Conflict of interest: None declared.

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## Author's reply

We would like to thank Arnott *et al* for their interesting comments. Needless to say, we all agree that great caution should apply when reporting positive or negative association studies because of issues such as sample size, cryptic population substructure, and phenotype misclassification. To this end, a transmission disequilibrium test (TDT) should always be performed to alleviate skepticism and doubts. It is true that a clear genetic heterogeneity emerges when examining NOD2 and toll-like receptor 4 (TLR4) carriage frequencies in patients with Crohn's disease in Europe, North America, and Asia. That Crohn's disease is a heterogeneous and polygenic disease is obvious in the light of recent genome wide screens. However, if multiple genes contribute to Crohn's disease, their relative impact may vary from one phenotype to another (or from one Crohn's

**Table 1** Number of patients studied and Toll-like receptor 4 Asp299Gly allele and genotype frequencies in Crohn's disease (CD), ulcerative colitis (UC), and healthy control (HC) populations from Leuven, Munich, and Edinburgh

	Patient numbers (allele frequencies)			Crohn's disease genotype frequencies		
	CD	UC	HC	Wild-type	Heterozygous	Homozygous
Leuven	334 (11.0%)	163 (10.0%)	139 (5.0%)	79.3	19.5	1.2
Munich	102 (7.0%)	98 (9.0%)	145 (4.0%)	85.0	15.0	0
Edinburgh	234 (10.3%)	246 (6.8%)	189 (8.8%)	79.5	19.7	0.8

HC allele frequencies differed between Edinburgh and Munich (*p* < 0.02, odds ratio 1.35 (confidence interval 1.1–4.5)) and those between Edinburgh and Leuven approached significance (*p* = 0.06).

disease to another Crohn's disease) but also, possibly, from one population to another (or from one ethnic background to another). In fact, the relative proportion of polymorphisms in susceptible genes that influence a specific phenotype may vary depending on the ethnic background. As highlighted by the Edinburgh group, the population attributable risk for NOD2 variants is significantly lower in Scotland and Ireland than in the rest of Europe or the USA, suggesting a much lower contribution of NOD2 variants (or perhaps of pattern recognition receptors (PRRs)) in Crohn's disease.<sup>1</sup> Interestingly, this is reinforced by their recent observation of a negative association between Crohn's disease and the TLR4 Asp299Gly polymorphism. High resolution haplotype mapping of TLRs and signalling molecules should allow us to discriminate the relative influence of these PRRs in Crohn's disease and ulcerative colitis across the world.

**D Franchimont**

Erasmus University Hospital, Brussels, Belgium

**S Vermeire**

University Hospital Gasthuisberg, Leuven, Belgium

**J Deviere**

Erasmus University Hospital, Brussels, Belgium

**P J Rutgeerts**

University Hospital Gasthuisberg, Leuven, Belgium

Correspondence to: Dr D Franchimont, Erasmus University Hospital, Lennik St, 808 Brussels, Belgium; denis.franchimont@ulb.ac.be

Conflict of interest: None declared.

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## Portopulmonary hypertension in cirrhosis: the pathogenetic challenge

We would like to thank Pellicelli (*Gut* 2004;**53**:1721) for his interest in our paper on portopulmonary hypertension in cirrhosis with refractory ascites (*Gut* 2003;**52**:1355–62).

We acknowledge that our proposal suggesting the role of endothelin 1 (ET-1) in the pathogenesis of portopulmonary hypertension is based on a small number of patients. However, the strong association between the elevated ET-1 levels in the pulmonary artery and the presence of portopulmonary hypertension in these patients would suggest that this is indeed the case. The confirmatory test would be a reduction in pulmonary pressure following blockade of endothelin receptors in the pulmonary circulation. However, this has not been reported to date. We also acknowledge that we did not measure the gradient of ET-1 across the pulmonary vascular bed, which is an important site for both production and clearance of ET-1. The fact that systemic arterial endothelin levels in patients with advanced cirrhosis are similar to those in control subjects<sup>1</sup> would suggest that there is no net production of ET-1 in the pulmonary circulation. Rather, the high levels of ET-1

delivered to the pulmonary circulation are being metabolised locally in these patients. Indeed, in experimental cirrhosis, there is evidence of increased expression of endothelin type B receptors in the pulmonary circulation, and these are responsible for clearance of ET-1.<sup>2</sup> Therefore, although our single measurement of ET-1 in the pulmonary artery does not reflect the net result of ET-1 metabolism in the pulmonary vascular bed, it does represent the amount of ET-1 that is delivered to the pulmonary artery, potentially causing pulmonary vasoconstriction.

With respect to measurement of ET-1 across the splanchnic circulation, there is already ample evidence in the literature to support increased production of ET-1 in the splanchnic circulation<sup>3,4</sup> as well as in the liver.<sup>5</sup> Therefore, it is reasonable to postulate that the increased ET-1 levels in the pulmonary artery, which is downstream from the splanchnic circulation, is largely derived from the increased production in that circulation.

Pellicelli's proposal of interleukin 6 (IL-6) being one of the mediators involved in the pathogenesis of portopulmonary hypertension in cirrhosis is an interesting one. IL-6 can increase platelet production in small muscular arteries and capillaries as well as enhancing platelet activation.<sup>6</sup> In addition, IL-6 promotes the coagulation cascade without affecting fibrinolysis, causing fibrin thrombi.<sup>6</sup> If this occurs in the pulmonary circulation, then pulmonary hypertension can develop. Similar to what has been reported in the literature,<sup>7</sup> Pellicelli and his group have found increased levels of IL-6 in patients with cirrhosis. However, it is not clear whether the increased plasma levels of IL-6 in cirrhosis are a non-specific inflammatory response in patients who are generally ill or part of a pathogenetic mechanism of some complication of cirrhosis. To date, there is no firm evidence that IL-6 is involved in the pathogenesis of either primary or secondary pulmonary hypertension. The challenge remains for us to find increased IL-6 levels in the pulmonary circulation in patients with portopulmonary hypertension before it can be implicated in the pathogenesis of this serious but uncommon complication of cirrhosis.

**F Wong**

Correspondence to: Dr F Wong, University of Toronto, 9EN/220, Toronto Hospital, 200 Elizabeth St, Toronto, Ontario, Canada; florence.wong@utoronto.ca

Conflict of interest: None declared.

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## Oesophageal entrapment of wireless capsule endoscopy in valvular patients

Wireless capsule endoscopy is an emerging new method of examining the small bowel. Its indications are currently widening, and occult and gastrointestinal bleeding of obscure origin, chronic diarrhoea and malabsorption syndromes, and suspicion of a small bowel neoplasm are now accepted indications.<sup>1</sup> Complications are rare, the main one being that it can become stuck in strictures or diverticulae inaccessible to flexible endoscopic retrieval.<sup>2</sup> The incidence in published series range from 1% to 5%, and all were managed with a surgical or endoscopic procedure.<sup>2,3</sup>

We present a complication seen in two of our patients, with no consequences to their health or management, but with an impact on examination accuracy and usefulness: oesophageal entrapment in an extra oesophageal vascular compression.

Patient 1 was a 74 year old man with a history of a prosthetic metallic mitral valve and congestive heart failure who had suffered three episodes of obscure overt gastrointestinal bleeding. In his community hospital he had undergone upper and lower endoscopy twice, radioisotope bleeding scans, magnetic resonance imaging angiography, and conventional angiography but the source of the gastrointestinal bleeding was not found. He needed oral iron supplementation but this did not correct his iron deficiency anaemia. Previous radiographic contrast studies of the small bowel had been normal. It was then referred to our unit for a capsule endoscopy examination. After the patient swallowed the capsule, we could see that without an apparent intrinsic stricture, the capsule was retained in the second third of the oesophagus for approximately four hours, progressing to the stomach after this time with no apparent manoeuvre or fluid ingestion (fig 1). The study could not be completed to the terminal ileum because the capsule batteries became exhausted at the level of the proximal ileum. Nevertheless, we could see three bleeding jejunal ulcers as the cause of his gastrointestinal bleeding.

Our second patient was a 72 year old woman with a mitral prosthetic valve and chronic auricular fibrillation. She had undergone upper and lower gastrointestinal endoscopy and mesenteric angiography because she had passed dark stools, with severe anaemia, on repeated occasions. A plain chest x ray showed marked cardiomegaly. She was referred to our centre for a small bowel capsule endoscopy examination. The capsule got stuck at a pulsate area in the distal oesophagus, staying there for up to three hours and passing afterwards without a specifically related cause (fig 2). The study



**Figure 1** The capsule was retained in the second third of the oesophagus for approximately four hours (the scan was taken nearly two hours after the obstruction).



**Figure 2** The capsule became stuck in the distal oesophageal, retained by an extrinsic stricture.

was also suboptimal because the patient had ingested some fluids and food that interfered with small bowel vision.

This complication of wireless capsule endoscopy has not previously been reported. An elongated aorta was probably the cause of extrinsic pulsate compression of the thoracic oesophagus in our first case, and a dilated auricular cavity in the second. In both patients upper endoscopy had been performed twice and no strictures were identified.

Although not a life threatening complication, it limited the procedure results in both cases, leading to suboptimal results. A plain chest x ray or accurate oesophageal radiographic contrast studies, previous to the wireless capsule endoscopy procedure, could have provided clues. In such patients, it is advisable to assure passage into the stomach of the capsule on x ray. If the capsule is retained, water could be given to the patient to drink followed by a repeated plain chest x ray to exclude oesophageal entrapment of the capsule. Nevertheless, in a previous report, capsule impaction at the cricopharynx was described and the solution adopted by clinicians was endoscopic placement of the capsule in the stomach.<sup>4</sup> This could be a feasible solution for patients in whom the capsule is struck in an extrinsic vascular stenosis in the oesophageal lumen.

**E Redondo-Cerezo, A Pérez-Sola, C Gómez, G Pérez-Vigara, J I Pérez-García, J Morillas, J A González-Martín**

Digestive Diseases Department, Gastrointestinal Endoscopy Unit, Hospital General Virgen de la Luz, Cuenca, Spain

Correspondence to: Dr E Redondo-Cerezo, Sección de Aparato Digestivo, Unidad de Endoscopias, Hospital General Virgen de la Luz, C/ Hermandad de Donantes de Sangre, 16002-Cuenca, Spain; eredondoc@yahoo.es

doi: 10.1136/gut.2004.051318

Conflict of interest: None declared.

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## Endogenous heparinoids in acute variceal bleeding

The risk of variceal bleeding in cirrhotics is associated with increasing liver dysfunction, larger varices, endoscopic red signs, and higher portal pressure. However, why bleeding occurs unpredictably and infrequently in individual patients is unknown.

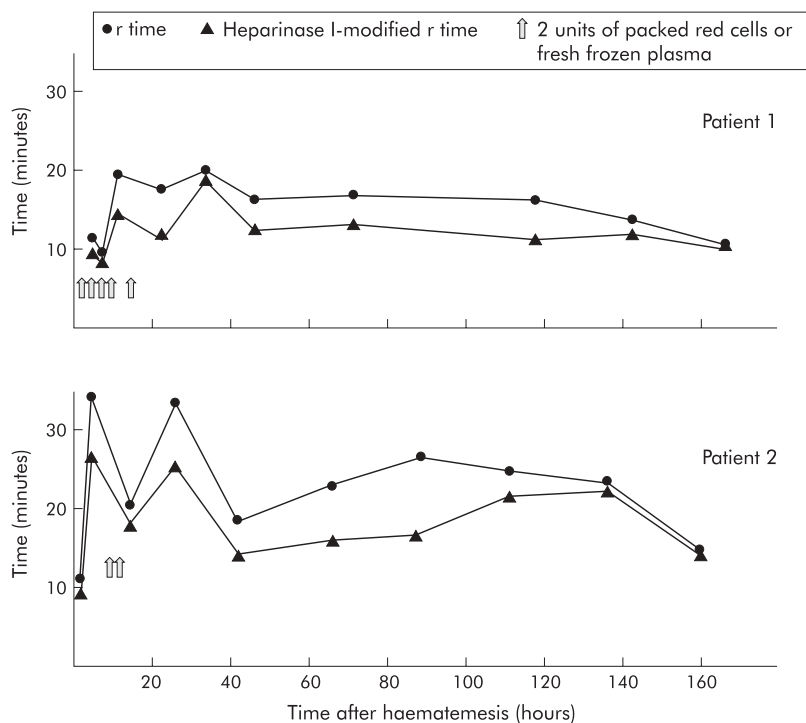
Bacterial infections occur in 35–66% of cirrhotics presenting with gastrointestinal bleeding.<sup>1</sup> We proposed a possible pathophysiological basis linking infection and variceal

bleeding via endotoxin induced endothelin release and subsequent portal pressure rise, combined with impaired platelet aggregation due to endotoxin induced nitric oxide and prostacyclin.<sup>2</sup> Infected cirrhotics demonstrate a heparin effect using heparinase I modified thromboelastography (TEG) and have anti-Xa activity.<sup>3,4</sup> Now we show similar findings in two cirrhotics during the course of acute variceal bleeding.

Patient 1 was male, 66 years old (Child-Pugh grade C), and patient 2 was female, 42 years old (Child-Pugh grade B), both with alcoholic cirrhosis. Both received endoscopic banding, intravenous terlipressin, and cefotaxime prophylactically as currently recommended.<sup>1</sup> Baseline bacterial screens were negative with no subsequent infections. Blood samples after informed consent were taken at baseline (before any therapy) and subsequently over seven days. Heparinase I modified and standard TEG (Haemoscope Corp., Skokie, Illinois, USA) were performed simultaneously using only calcium activated citrated blood from the same sample 90 minutes after venepuncture<sup>5</sup>: a heparin effect was defined as an improvement in r time, k time, and  $\alpha$  angle occurring together. Anti-Xa was assessed by chromogenic (Sigma Diagnostics, Poole, Dorset, UK) and clotting assays (Diagnostic Reagents, Thame, Oxford, UK).

A heparin effect was detected between one hour (patient 2) and six hours (patient 1) after the initial bleeding episode and persisted for 6–7 days, not fully corrected by fresh frozen plasma and/or red blood cells (fig 1) given during the first 24 hours. In patient 1, anti-Xa activity was positive during the same time span in which there was a heparin effect.

Evaluated TEG parameters were “r time” (time for the clot to start forming), “k time” (time between the TEG trace reaching 2 mm and 20 mm), and “ $\alpha$  angle” (the slope drawn



**Figure 1** Comparison of standard and heparinase I modified thromboelastography with respect to r time in two patients with variceal bleeding.



from the r to the k value). These worsened over time: in patient 1, r time 11 minutes (four hours after the bleeding episode) to 18.8 minutes (six hours later); k time from 2.7 minutes to 7.3 minutes; and  $\alpha$  angle from 53.8° to 28.7°. In patient 2, r time increased from 11.2 minutes (one hour after the first haematemesis) to 33.8 minutes (two hours later); k time from 3.1 minutes to 10.7 minutes; and  $\alpha$  angle from 50.8° to 17.8°. These values slowly returned to baseline concomitantly with disappearance of the heparin effect after 6–7 days.

Routine coagulation parameters (prothrombin time, activated partial thromboplastin time) did not show any correlation with worsening TEG parameters or the heparin effect.

The presence of endogenous heparinoids in cirrhotic patients with acute variceal bleeding is clearly demonstrated. There was no evidence of infection but the antibiotic prophylaxis possibly prevented or treated infection. Neither patient experienced early rebleeding. The heparin effect was documented shortly after the beginning of the haemorrhage and disappeared over five days, over the same time course of antibiotic therapy. This was also seen by Montalto and colleagues.<sup>3</sup> The absence of a demonstrable heparin effect at the beginning of bleeding and appearance thereafter could suggest that bleeding is a cause of its occurrence, and not the other way round. However, citrated blood may mask an initial less severe heparin effect and this needs to be evaluated compared with native blood. The heparin effect could influence continued variceal bleeding or early rebleeding. It is possible that the heparin effect might be worse in the absence of antibiotics. This phenomenon deserves wider study, particularly as bacterial infection has been linked to failure to control variceal bleeding and early rebleeding, in a randomised study of prophylactic antibiotics.<sup>6</sup>

**U Thalheimer, C Triantos, D Samonakis, D Patch, A K Burroughs**

Liver Transplant and Hepatobiliary Medicine Unit, Royal Free Hospital, London, UK

**A Riddell, D Perry**

Haemophilia Unit, Royal Free Hospital, London, UK

Correspondence to: Professor A K Burroughs, Liver Transplant and Hepatobiliary Medicine Unit, Royal Free Hospital, Pond St, London NW3 2QG, UK; Andrew.Burroughs@royalfree.nhs.uk

doi: 10.1136/gut.2004.051474

Conflict of interest: None declared.

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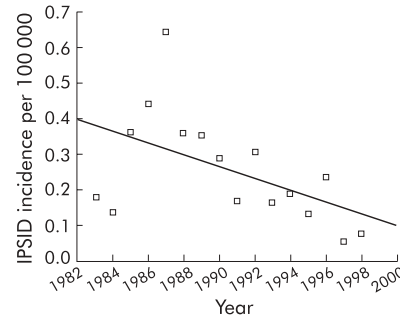
**Changing epidemiology of IPSID in Southern Iran**

Some 40 years ago physicians in the Middle East noted a high incidence and prevalence of upper small intestinal lymphoma.<sup>1–3</sup> Later this condition was found to be associated with malabsorption as well as the presence of alpha heavy chain proteins.<sup>2–7</sup> The disease was named by the WHO as “immunoproliferative small intestinal disease” (IPSID).<sup>6</sup> One of the earliest reports of IPSID was from our centre.<sup>3,4</sup> This study was designed to confirm the trend in the epidemiology of IPSID over the past 25 years in our medical centre. In a retrospective study (March 1974 to March 1999), we reviewed pathology reports from all surgical pathology laboratories in the province of Fars located in Southern Iran.

All reports, which were labelled as IPSID, were reviewed by one of the authors. Cases were grouped into five year intervals according to the date of the initial diagnosis and five year age groups. Age specific rates were calculated using midperiod population denominators for each age group, and summary age adjusted incidence rates were calculated by direct standardisation using the world standard population.<sup>8</sup>

During this 25 year period, more than 500 000 surgical pathology reports were recorded. There were 5421 gastrointestinal tract cancers of which 2326 (43%) were gastric cancers, 1398 (26%) colonic cancers 1161 (21%) oesophageal cancers, and 536 (10%) small bowel cancers. Of the small bowel cancers, 161 (30%) cases were IPSID. This composed 3% of all gastrointestinal cancers in this period.

Among the 161 IPSID cases, 98 (61%) were males with a mean age of 31.74 (SD 14.94) years and 63 (39%) were females (mean age 26.85 (8.88)). The standardised rate ratio (95% confidence interval) of males to females in the study was 1.39 (1.26, 1.69), which represents a higher incidence of IPSID in males. Almost all cases were village



**Figure 1** Incidence rate of immunoproliferative small intestinal disease (IPSID) in the Fars province, Iran, from 1983 to 1999.

dwellers or those who had recently immigrated to large cities from their villages. Age specific rates and absolute frequency of IPSID in males and females are shown in table 1. The disease had its highest incidence in the third decade of life in both sexes. There has been a persistent decrease in the incidence of IPSID since 1986, as show in fig 1.

The sharp decrease in the incidence of IPSID in the period 1978–1983 coincided with the time of revolution and the Iraq-Iran war, which caused instability in all organisations. The incidence of IPSID has decreased over the past 15 years ( $r^2 = 0.26$ ,  $t(14) = -2.25$ ,  $p = 0.04$ ).

IPSID was once the most common small intestinal malignancy in the Middle East.<sup>1–6</sup> Early infectious stress in infancy and chronic antigenic stimulation in the earlier part of life along with genetic factors are probably important in the pathogenesis of IPSID.<sup>7</sup> In our series of 161 patients with IPSID, we observed a dramatic decrease in the incidence of the disease over the past decade.

After the Islamic revolution in Iran, improving sanitation in villages was one of the priorities of the many health strategies in Iran. Access to sanitary drinking water in rural areas increased from 35% before 1988 to 80% a decade later.<sup>9</sup> Vaccination programmes increased dramatically after the Islamic revolution, reaching more than 90% of children.<sup>9</sup> Local health facilities increased dramatically during the first two decades after the revolution.<sup>9</sup>

**Table 1** Age specific rates (ASR) and absolute frequency of immunoproliferative small intestinal disease in males and females in different age groups in the Fars province, Iran, from 1974 to 1999

Age group (y)	Total	ASR	Males	ASR	Females	ASR
0–4	2	0.02	1	0.02	1	0.02
5–9	6	0.05	6	0.1	0	0
10–14	5	0.05	4	0.08	1	0.02
15–19	14	0.17	9	0.21	5	0.12
20–24	35	0.54	14	0.43	21	0.64
25–29	35	0.59	17	0.62	18	0.56
30–34	11	0.25	7	0.31	4	0.18
35–39	16	0.43	11	0.58	5	0.27
40–44	11	0.38	8	0.54	3	0.21
45–49	10	0.42	7	0.57	3	0.26
50–54	7	0.32	7	0.61	0	0
55–59	5	0.28	3	0.31	2	0.25
60–64	2	0.12	2	0.22	0	0
>65	2	0.13	2	0.25	0	0
Total	161	0.21	98	0.25	63	0.170

Age specific rate per 100 000 population in each age group.

We postulate that improvement in health in general and decreasing childhood gastroenteritides in particular has resulted in a decrease in the incidence of IPSID. This report highlights the almost complete disappearance of a malignant disease from a region where it was once very common. This is probably related to changes in environmental factors, decreasing exposure to infectious agents.

**K B Lankarani, S M Masoompour, M B Masoompour, R Malekzadeh, S Z Tabei, M Haghshenas**

Gastroenterology and Hepatology Research Center, Shiraz University of Medical Sciences, Shiraz, Islamic Republic of Iran

Correspondence to: Dr K B Lankarani, Gastroenterology and Hepatology Research Center, Shiraz University of Medical Sciences, PO Box 71345-1414, Shiraz, Islamic Republic of Iran; lankaran@sums.ac.ir

doi: 10.1136/gut.2004.050526

Conflict of interest: None declared.

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## Microscopic (collagenous and lymphocytic) colitis triggered by food allergy

Collagenous and lymphocytic colitis<sup>1,2</sup> are rare<sup>3</sup> diseases of unknown aetiology but several issues,<sup>1-3,5</sup> in particular the good response to budesonide,<sup>7</sup> are suggestive of immunopathology. Patients have watery

diarrhoea without abnormal findings on colonoscopy<sup>1-5</sup> but with increased numbers of intraepithelial lymphocytes, mast cells, and eosinophils<sup>3</sup> on histological examination.

We report six patients seen between 1993 and 1999 who were first diagnosed as having collagenous/lymphocytic colitis. Signs of allergy entailed a work up for food allergy. (table 1).

All patients were investigated with skin prick testing, total and allergen specific IgE with food, and environmental allergens. Excretion of urine methylhistamine (UMH) was measured<sup>8</sup> on a normal and hypoallergenic potato-rice diet. Colonoscopy with endoscopically guided segmental lavage for intestinal IgE was carried out<sup>9</sup> and biopsies were investigated by routine pathology (haematoxylin-eosin), immunohistochemistry for eosinophil peroxidase, and amount of eosinophil cationic protein and tryptase<sup>9</sup> in the whole biopsy. Clinical activity was mainly assessed by number of stools/day<sup>7</sup> and the Karnofsky index for general performance.

After allergen identification, all patients were counselled on elimination of the allergen, except for one case where no allergen was identified. In this case, in a second patient with multiple sensitisations, and in a third with allergy to basal foods, additional cromolyn therapy was initiated. A trial of hypoallergenic diet and subsequent controlled addition of food with low allergenicity was performed. Additional antihistaminergic therapy (fexofenadine) was recommended as supplementary therapy for periods of exacerbation.

All patients were followed prospectively every 12 months after diagnosis (outpatient clinic and structured telephone interview) and all had symptom reduction in terms of stool frequency and consistency (table 2). General performance was completely restored in four patients and improved in one. The remaining patient is still very restricted in his activities due to incapacitating coronary artery disease but his gastrointestinal symptoms are tolerable.

Another patient was not willing to undergo colonoscopy for lavage and allergen identification, nor willing to quit smoking. His stools normalised after a six month course of cromolyn but he still suffers from bouts of diarrhoea during stress. He considers his general performance as good.

Histology was available for one patient before and after therapy. After dietary elimination, eosinophilic infiltrate was markedly less dense and degranulated.

The mechanisms of diarrhoea in collagenous colitis include a pronounced diffusion barrier with diminished net absorption of sodium and chloride ions.<sup>6</sup> Allergens could induce increased eosinophil infiltration and

**Table 2** Number of stools per day (Baerts score<sup>7</sup>) before and after therapy

Sex	Age (y)	Before diagnosis	After diagnosis
F	49	5-10	1-2
M	45	5	0
M	46	4-6	0-1
M	52	10	1
M	71	4	1
F	59	5	1

enhance transforming growth factor  $\beta^{10}$  with increased collagen deposition. Eosinophils are highly susceptible to steroids which may explain the good response of collagenous colitis to budesonide.<sup>4</sup>

In summary, a subgroup of patients with microscopic colitis suffer from food allergy. Further work up for allergy is sensible in those patients with a history of atopic disease or blood/tissue eosinophilia. Allergen elimination can decrease or abolish the need for medication. Antiallergic therapy can be added to the therapeutic regimen.

### M Weidenhiller

Clinic for Internal Medicine A, Ernst-Moritz-Arndt-University, Greifswald, Germany, and Department of Medicine I, University of Erlangen-Nürnberg, Erlangen, Germany

### S Müller

Institute for Pathology, University of Erlangen-Nürnberg, Erlangen, Germany

### D Schwab, E G Hahn, M Raitchel

Department of Medicine I, University of Erlangen-Nürnberg, Erlangen, Germany

### S Winterkamp

Department of Medicine I, University of Erlangen-Nürnberg, Erlangen, Germany, and Fachkrankenhaus Kloster Grafschaft, Pneumology and Weaning Centre, Schmallenberg, Germany

Correspondence to: Dr M Weidenhiller, Clinic of Internal Medicine A, Friedrich-Loeffler-Str 23 A, DE-17489 Greifswald, Germany; michael.weidenhiller@uni-greifswald.de

doi: 10.1136/gut.2004.049767



Conflict of interest: declared (the declaration can be viewed on the Gut website at <http://www.gut.com/supplemental>).

**Table 1** Patient characteristics

Sex	Age (y)	Follow up (y)	Atopy	Signs of allergy	Diagnostic markers suggestive of allergy	Identified allergen
F	49	5	+	Improvement by hypoallergenic diet	Eos in bx	Egg, pollen, housedust mite
M	45	5	-	Urticaria, improvement by cromolyn	UMH	-
M	46	5	-	Pansinusitis	Eos in bx	Spices, moulds, flours, celery, nuts, milk, maize, rice, apple, celery, soy
M	52	3	-	IgG deficiency	Eos in bx	Maize starch
M	71	9	+	Prick	Prick, RAST	Milk, egg, soy
F	59	4	+	Prick	UMH	Ananas

Eos in bx, eosinophils in colon biopsy; UMH, urine methylhistamine.

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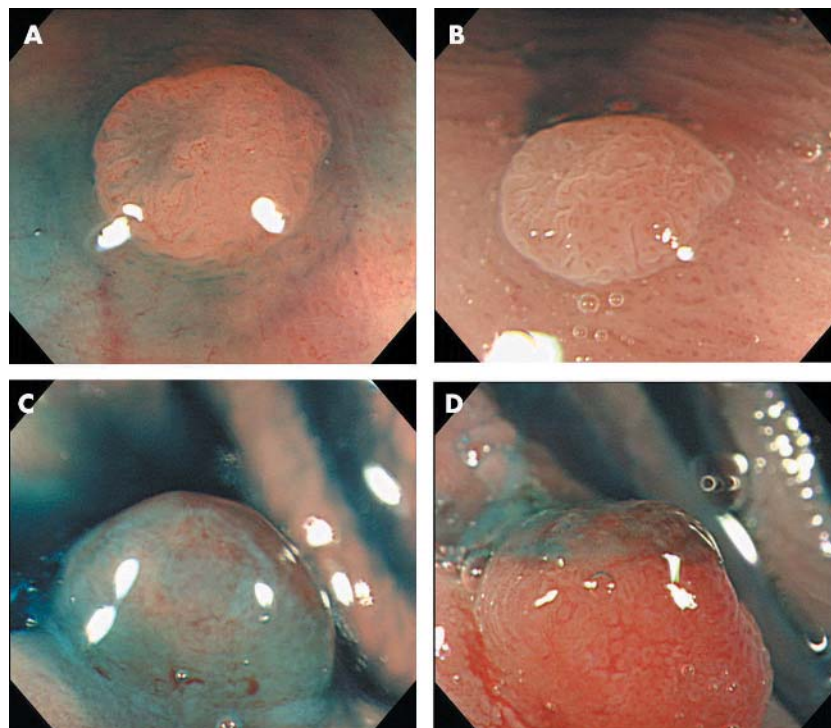
### Acetic acid spray in colonoscopy: an alternative to chromoendoscopy

We read with interest the article by Rutter *et al* (*Gut* 2004;53:256–60) and the letter in response to this article by Hata *et al* (*Gut* 2004;53:1722). Rutter demonstrated the advantage of magnifying chromoendoscopy using indigo carmine for detection of dysplasia compared with conventional colonoscopy without dye spray by back to back colonoscopy in patients with longstanding ulcerative colitis.

Hata *et al* discussed the characteristics and correct selection of dyes. In particular, Hata *et al* emphasised that there are two types of dye spraying: the contrast method in which dye is used solely to contrast the irregularity of the surface, and the staining method in which dyes such as crystal violet and methylene blue are used to stain the colonic mucosa. The latter technique provides more detailed structure of neoplastic as well as non-neoplastic colonic mucosa, which may contribute to more precise diagnosis of dysplasia and inflammatory change than the contrast method.

However, as Hata *et al* pointed out, a disadvantage of the staining method is that it is time consuming. Usually, it takes two or three minutes to stain one region. Therefore, it would be beneficial if there were an agent that demonstrated the fine structure of the colonic mucosa without a delay.

Recently, we have introduced acetic acid spray in screening colonoscopy to visualise the fine structure of colonic neoplasia, in which approximately 5 ml of 2% acetic acid solution is sprayed towards the targeted lesion in the same manner as for indigo carmine. The advantages of using acetic acid spray as a staining method are as follows. Firstly, the fine structure of the mucosa can be demonstrated immediately (fig 1A, 1B).



**Figure 1** (A) Magnifying chromoendoscopy using the contrast method. Indigo carmine spray reveals a neoplastic pattern. However, the pit pattern cannot be observed in some parts of the tumour. (B) After acetic acid spray, the detailed structure of the entire surface is immediately demonstrated more meticulously than with the contrast method. (C) Polyp covered with mucus. Dye spray using indigo carmine fails to reveal the surface structure because of the mucus. (D) After acetic acid spray, the mucous has been clearly removed and the surface of the polyp can be evaluated.

Therefore, it reduces the time for examination, especially in patients with multiple lesions. Secondly, acetic acid effectively removes surface mucous material that interferes with magnifying observations (fig 1C, 1D). Lastly, acetic acid is less expensive.

We agree with Hata *et al* that it is essential to understand the various methods of dye spray and to apply them appropriately, according to the situation. Here, we advocate acetic acid spray as an alternative to dye spray for enhancing the fine structure of the mucosa. Hata *et al* titled their letter "To dye or not to dye. That is beyond question!" We would like to add "To spray dye or to spray acetic acid. That is our question!"

**Y J Kawamura, K Togashi, J Sasaki, F Konishi**  
Jichi Medical School, Saitama, Japan

Correspondence to: Dr Y J Kawamura, Jichi Medical School, 1-847, Amanuma-cho, Omiya-ku, Saitama-shi, Saitama 3308503, Japan; kawamura@omiyajichi.ac.jp

Conflict of interest: None declared.

### Infliximab failure in cap polyposis

Cap polyposis is a rare condition that predominantly affects the rectosigmoid with distinctive clinicopathological features. The common symptoms are mucoid and bloody diarrhoea with abdominal pain and tenesmus. At endoscopy, polyps are red, sessile, and located at the apices of enlarged transverse mucosal folds with a normal

intervening mucosa. Microscopic features include elongated hyperplastic looking glands with a mixed inflammatory infiltrate in the lamina propria. A cap of fibrinopurulent exudate covers the polyps. Treatment of this condition remains empiric. Metronidazole and steroids have been effective in some cases. Symptoms are often relieved by polypectomy but rectosigmoid resection may be required to control diarrhoea.

Some years ago, we reported in *Gut* on a 52 year old woman who needed sigmoid resection for cap polyposis.<sup>1</sup> Following surgery, she did well until 1998 when she again complained of abundant mucoid diarrhoea with severe postprandial abdominal pain requiring daily antispasmodic therapy. Endoscopy with histology displayed the characteristic features of recurrent cap polyposis in the rectum (fig 1). This was again refractory to multiple therapies, including several courses of mesalamine, antibiotics, steroids, and laser photocoagulation of polyps. At that time, we were aware of a case of cap polyposis that was successfully treated with infliximab.<sup>2</sup> This was a 36 year old woman who had a one year history of cap polyposis and who experienced complete clinical, endoscopic, and histological remission following four infliximab infusions at eight week intervals. This encouraging observation led us to treat our patient with infliximab. Two infusions of infliximab 5 mg/kg were administered at four week intervals. In order to gain some insight regarding the potential involvement of tumour necrosis factor  $\alpha$  (TNF- $\alpha$ ) in this condition, TNF- $\alpha$  mRNA was measured in the





**Figure 1** Typical aspect of cap polyposis recurring in the rectum after sigmoid resection. Polyps are red, sessile, and covered with an exudate.

rectal mucosa using real time polymerase chain reaction before and after treatment, and compared with control values. Unfortunately, no clinical or endoscopic improvement occurred following infliximab infusions. TNF- $\alpha$  levels in the mucosa were not different compared with controls before and after treatment.

The reason for the discrepancy between this failure and the spectacular improvement observed by Bookman and colleagues<sup>2</sup> is unclear. Our patient suffered from this condition for more than 10 years and may have had a more refractory form of the disease. One should also remember that in the case described by Bookman and colleagues, no control was available and spontaneous regression of cap polyposis has already been observed.<sup>3,4</sup> The pathogenesis of cap polyposis remains unclear. Our data do not support the hypothesis that TNF- $\alpha$  plays a role in the pathogenesis of cap polyposis. An infectious or ischaemic aetiology has been suspected. Histological features similar to cap polyposis have been described in other disorders where mucosal prolapse is the underlying mechanism such as solitary rectal ulcer syndrome or prolapsed colostomies.<sup>5</sup> It has therefore been suggested that abnormal colonic motility may be an important aetiological factor.

V Maunoury, M Breisse, P Desreumaux, L Gambiez, J-F Colombel

Clinique des Maladies de l'Appareil Digestif et al de la Nutrition, Centre Hospitalier Universitaire Lille, Lille, France

Correspondence to: Professor J-F Colombel, Clinique des Maladies de l'Appareil Digestif et al de la Nutrition, Centre Hospitalier Universitaire Lille, CH et al U Lille, 59037, Lille, France; jfcolombel@chru-lille.fr

doi: 10.1136/gut.2004.053686

Conflict of interest: None declared.

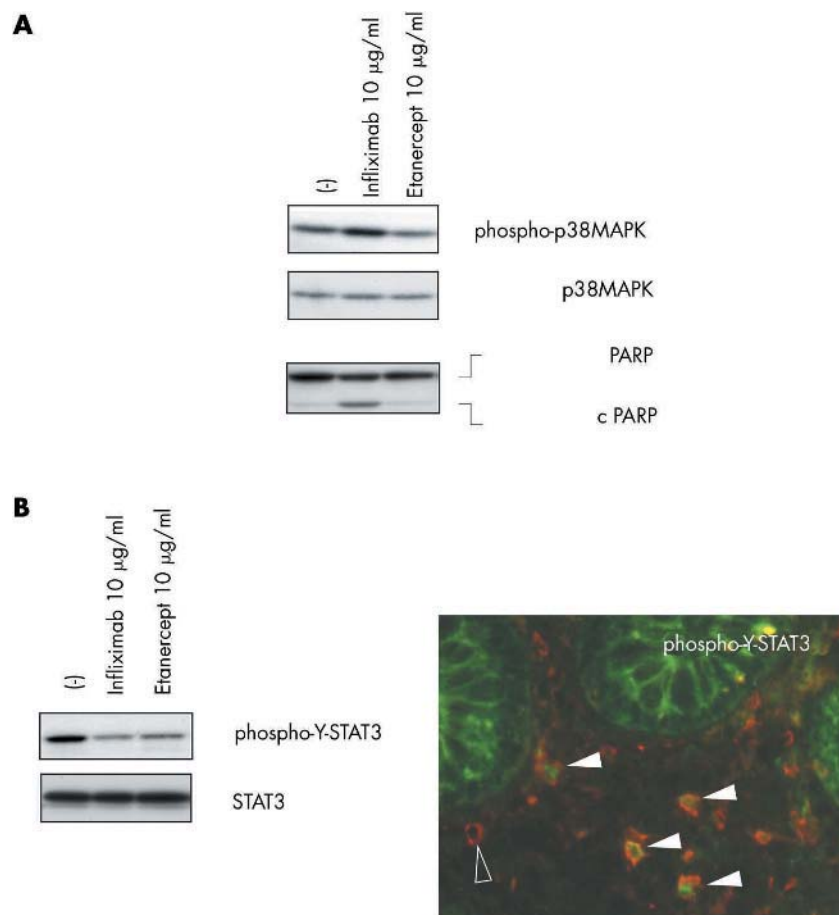
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## Differential modulation of p38 mitogen activated protein kinase and STAT3 signalling pathways by infliximab and etanercept in intestinal T cells from patients with Crohn's disease

There is growing evidence that the efficacy of anti-tumour necrosis factor  $\alpha$  (TNF- $\alpha$ ) therapies in Crohn's disease (CD) may critically

depend on the binding of the transmembrane precursor of TNF- $\alpha$  (mTNF- $\alpha$ ), thus eliciting complex intracellular signalling events, a process described as "reverse signalling".<sup>1–3</sup> In their recent paper (*Gut* 2004;**53**:70–7), Di Sabatino *et al* showed that infliximab reverted defective peripheral and lamina propria lymphocyte apoptosis in CD patients via a caspase dependent mechanism, further corroborating the findings of previous studies.<sup>1–4</sup> It has also been suggested that failure of another TNF binding agent, etanercept (Enbrel; a recombinant TNFR2:Fc fusion protein), to induce peripheral and lamina propria lymphocyte apoptosis,<sup>4</sup> provides a possible molecular explanation for the lack of efficacy of etanercept in a randomised placebo controlled trial in active CD.<sup>5</sup>



**Figure 1** Intestinal CD4<sup>+</sup> T cells were obtained from colonic biopsies from five patients with Crohn's disease (median age 34 years (range 18–49)) with an established diagnosis of Crohn's disease based on histopathological and endoscopic criteria. All patients had active disease at inclusion and were treated with 5-ASA (2–4 g/day, five patients), prednisone (20 mg/day, one patient), and azathioprine (150–200 mg/day, four patients). None of the patients had received anti-TNF- $\alpha$  treatment. Neither antigen nor feeder cells were added to the intestinal T cells cultures, which consisted of more than 97% CD3<sup>+</sup>/CD4<sup>+</sup> T cells. (A) Infliximab, but not etanercept, activated p38 $\alpha$  and induced apoptosis in intestinal T lymphocytes from Crohn's disease patients. Levels of (phosphorylated) p38 mitogen activated protein kinase (MAPK) and PARP in in situ activated intestinal T lymphocytes were investigated by western blot 24 hours after stimulation with the respective TNF- $\alpha$  binding agent, as described previously.<sup>3</sup> Data are representative of experiments performed in all patients, n = 5. (B) Signal transducer and activator of transcription 3 (STAT3) was activated in CD4<sup>+</sup> cells in the intestinal mucosa in Crohn's disease patients in vivo and downregulated by both infliximab and etanercept in intestinal T cells from Crohn's disease patients in vitro. Western blot and immunofluorescence staining were performed as described previously.<sup>3</sup> Filled arrowheads, cells positive for phospho-Y-STAT3 (anti-phospho-Y-STAT3, 1/100; Cell Signaling Technology) and CD4 (anti-CD4, 1/500; BD PharMingen, San Diego, California, USA); open arrowhead, CD4 immunoreactivity only (n = 5 for immunofluorescence and western blot; representative result for all experiments).

However, the authors do not discuss other signalling pathways that are activated via ligation of transmembrane TNF- $\alpha$  by infliximab (for example, we have shown that infliximab also transiently activates p38 mitogen activated protein kinase (MAPK) in monocytes in vitro and in the lamina propria of CD patients in vivo<sup>3</sup>). Responders and non-responders to infliximab differ in the pattern of mucosal p38MAPK target phosphorylation, but not caspase-3 activation, further emphasising the complex modulation of intracellular signalling pathways beyond mere neutralisation of sTNF- $\alpha$ .<sup>6</sup> To show if these signalling pathways are also activated in primary T cells, we analysed the influence of infliximab and etanercept on p38MAPK activation and apoptosis in an established model of non-transformed in situ activated T lymphocytes.<sup>7,8</sup>

According to the findings of van den Brande *et al.*,<sup>4</sup> we observed PARP cleavage as a molecular hallmark of apoptosis in cultures grown with infliximab (fig1A) but not in the presence of etanercept. Whereas no increase in phosphorylated p38MAPK could be detected after etanercept stimulation, significant activation (that is, dual phosphorylation) of p38MAPK 24 hours after infliximab treatment was observed in 4/5 of the cell lines derived from CD patients (fig1A).

We have demonstrated previously that constitutive tyrosine phosphorylation of the transcription factor signal transducer and activator of transcription 3 (STAT3) may represent a specific feature of intestinal T cells from Crohn's disease.<sup>9</sup> Tyrosine phosphorylated STAT3 can be found in CD4+ cells in the inflamed mucosa, as shown by immunofluorescence analysis (fig 1B). Surprisingly, infliximab and etanercept were able to reduce STAT3 phosphorylation in T cells from CD patients (cultured with interleukin (IL)-2 and IL-4) to a similar extent (fig 1B). It is tempting to speculate that the previously reported downregulation of interferon  $\gamma$ /granulocyte macrophage-colony stimulating factor by both infliximab and etanercept is also involved in the decrease in STAT3 tyrosine phosphorylation as both cytokines are potent inducers of STAT3 activation via Janus kinases. Mechanistically, this common action of infliximab and etanercept might be due to neutralisation of an autocrine loop of constitutively released sTNF- $\alpha$ . Although the findings suggest that inhibition of cytokine dependent inducible STAT3 phosphorylation could be dispensable for therapeutic efficacy in CD, aberrant constitutive STAT phosphorylation in T lymphocytes may still have an important modulatory role in chronic intestinal inflammation.<sup>8</sup>

These observations corroborate the hypothesis that TNF- $\alpha$  binding agents may exert distinct functions on lymphocyte activation and survival, either by "reverse signalling" via binding of mTNF- $\alpha$  resulting in p38MAPK activation and apoptosis or by neutralisation of sTNF- $\alpha$ , which in this model may serve to inhibit STAT3 signalling. The individual properties of TNF- $\alpha$  blockers to induce either of these complex molecular actions may be differentially responsible for therapeutic success or failure in chronic inflammatory disorders such as rheumatoid arthritis, psoriasis, or CD. In CD, ligation of mTNF- $\alpha$ , subsequent apoptotic processes, and MAPK signalling seem to be critically required. Molecular dissection of mTNF- $\alpha$  apoptotic and non-apoptotic signalling may have

important implications for the design of future therapeutic strategies in inflammatory bowel disease.

#### P Rosenstiel

Institute of Clinical Molecular Biology, University Hospital Schleswig-Holstein, Campus Kiel, Kiel, Germany

#### J Agnholt, J Kelsen

Department of Medicine V, Aarhus University Hospital, Aarhus, Denmark

#### V Medici

Institute of Clinical Molecular Biology, University Hospital Schleswig-Holstein, Campus Kiel, Kiel, Germany

#### G H Waetzig, D Seegert

Conaris Research Institute, Kiel, Germany

#### S Schreiber

Institute of Clinical Molecular Biology, University Hospital Schleswig-Holstein, Campus Kiel, Kiel, Germany

Correspondence to: Professor S Schreiber, Institute of Clinical Molecular Biology, University Hospital Schleswig-Holstein, Campus Kiel, Schittenhelmstrasse 12, 24105 Kiel, Germany; s.schreiber@mucosa.de

Conflict of interest: None declared.

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## Author's reply

We thank Rosenstiel *et al* for their interest in our paper regarding the role of the chimeric anti-tumour necrosis factor (TNF)  $\alpha$  antibody, infliximab, in reverting defective lamina propria T cell apoptosis in Crohn's disease via a caspase dependent pathway (*Gut* 2004;**53**:70–7).

In their interesting letter, Rosenstiel *et al* investigate the complex TNF- $\alpha$  blocker machinery in Crohn's disease by analysing the influence of infliximab and recombinant TNF receptor/immunoglobulin G fusion protein, etanercept, on p38 mitogen activated

protein kinase (MAPK) activation, apoptosis, and signal transducer and activator of transcription 3 (STAT3) tyrosine phosphorylation in lamina propria T cells from active Crohn's disease patients. Infliximab, but not etanercept, has been shown to result in p38MAPK activation and apoptosis, while both of these agents were able to reduce STAT3 phosphorylation in Crohn's disease lamina propria T cells.

If data on the differences in mucosal T cell apoptosis inducing capacity of infliximab and etanercept are in keeping with the results obtained by van den Brande and colleagues,<sup>1</sup> the observation that infliximab induces activation of a proinflammatory MAPK does not tally with the evidence that the guanlylhydrazone c-Jun N terminal kinase/p38 inhibitor CNI-1493 induces clinical improvement in steroid resistant Crohn's disease patients.<sup>2</sup> The reason for this discrepancy probably lies in the complex and dichotomous role of p38MAPK in inflammatory signal transduction. p38MAPK inhibition is efficacious in chronic inflammatory disease but not in acute experimental colitis, where it causes adverse effects,<sup>2,3</sup> and it shares differential actions in different T cell subpopulations.<sup>4</sup> In naive T cells, in fact, the p38 inhibitor SB203580 inhibits proinflammatory cytokine production whereas in T helper type (Th) 1 cells it does not affect TNF- $\alpha$  release but strongly impairs anti-inflammatory interleukin 10 production, thus tilting the balance between Th1 and Th2 cytokines to the former.<sup>4</sup>

Since several lines of evidence have also suggested the proapoptotic role of the p38 pathway,<sup>5</sup> Waetzig and colleagues<sup>4</sup> hypothesised that the increased phosphorylation of the p38MAPK downstream effector ATF-2, found after infliximab treatment only in responder Crohn's disease patients, could enhance infliximab induced apoptosis of immune cells in this subgroup of patients. However, the similar proportion of lamina propria immune cell apoptosis after infliximab treatment in both responders and non-responders suggests that differences in signal transduction downstream of p38MAPK are not related to infliximab induced immune cell apoptosis.<sup>4</sup>

Disturbances in STAT signalling pathways, which transduce the immunomodulatory messages of most of cytokines/cytokine receptors, have been shown to be involved in the pathogenesis of Crohn's disease. In particular, STAT3 and STAT4 proteins are constitutively activated in lamina propria CD4+ T cells from active Crohn's disease patients.<sup>6</sup> The finding of Rosenstiel *et al* that both infliximab and etanercept are able to reduce in vitro STAT3 tyrosine phosphorylation of Crohn's disease lamina propria T cells is probably related to the capacity of both of these agents to neutralise soluble TNF- $\alpha$ . Interestingly, the absence of quantitative differences in the potential of infliximab and etanercept in downregulating STAT3 signalling is consistent with the data of van den Brande and colleagues<sup>1</sup> who have shown that both drugs can neutralise soluble TNF- $\alpha$  to a similar extent.

Taken together, these findings suggest that both the apoptotic and non-apoptotic intracellular signalling pathways underlying the therapeutic benefit of anti-TNF- $\alpha$  strategies in Crohn's disease are multifaceted and more complex than initially thought. Dissecting the MAPK signalling cascades that are selectively activated in the abnormal immune response



of inflammatory bowel disease is critical in the identification of selective targets and development of new and rational therapies for the treatment of Crohn's disease.

**A Di Sabatino, R Ciccocioppo, R Morera,  
G R Corazza**

Gastroenterology Unit, IRCCS Policlinico S. Matteo,  
University of Pavia, Pavia, Italy

Correspondence to: Dr A Di Sabatino,  
Gastroenterology Unit, IRCCS Policlinico S Matteo,  
University of Pavia, Pavia, Italy;  
a.disabatino@smatteo.pv.it

Conflict of interest: None declared.

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## BOOK REVIEW

### Small and Large Intestine

Edited by G Lichtenstein, G Wu. Pennsylvania: Mosby, 2003, £62.99, pp 364. ISBN 0323018955

The *Small and Large Intestine*, one of four volumes in this series named the *Requisites in Gastroenterology* edited by Anil Rustgi of the University of Pennsylvania, provides a refreshing readable overview. While successfully avoiding entrenchment in detailed consideration of the scientific literature, it provides a mainstream viewpoint of the major issues. It is presented in a logical and clear manner aimed at achieving an understanding at a basic level which will provide the reader with a good platform to focus on specific areas with more in depth study. In

this series, Rustgi moves away from an account of the substantial gastroenterological literature which often includes clinical trials with apparently contradictory conclusions that may lead to controversies unwanted by the novice. Rather than being expected to weigh up the evidence, the student is more usually interested in the general viewpoint of the main body of experts. This series avoids the comprehensive consideration of the literature often found in the larger reference texts and concentrates on the delivery of practical guidance and the acquisition of a general grasp of the subject, particularly for physicians in training and medical students.

Each of the 13 chapters have well defined formats, orientating the reader with a brief initial chapter outline and general introduction to the subject. The majority then discuss the epidemiology and pathophysiology before providing a more detailed account of clinical evaluation and treatment. Considerable attention is given to pragmatic clinical management issues cutting through much of the academic detail to provide practical information, useful to those considering the day to day issues of gastroenterology. For example, in the Crohn's disease chapter, there are two pages dedicated to pathogenesis, five to clinical assessment and diagnosis, followed by 16 pages on therapy of which only one relates to surgery, and that includes a table (clearly we are heading in the right direction with therapy!). However, nutritional therapy is given very little mention, which may reveal subtle differences in the character of medical gastroenterology between the USA and Europe.

Most chapters stay well within general dogma, including the most important, latest, and robust advances in knowledge; a few have a particular emphasis. The "irritable bowel syndrome" chapter develops the psychosocial/psychiatric approach to patients to a degree that might reflect the chapter author's own clinical experience as a professor of psychiatry and medicine. It nevertheless addresses the other pathogenic aspects of irritable bowel syndrome, although promotes rather strict Rome criteria to the diagnosis which perhaps does not describe the full range of irritable bowel syndrome patients seen in the average outpatient clinic and is of more value if a well defined group of patients is required, such as for conducting clinical trials. A positive diagnosis based on irritable bowel syndrome symptoms is also recommended. This should be tempered with caution as there are many pitfalls associated with this particular group of patients, particularly for those less experienced in this field of practice who are the target audience for this textbook. The chapters on "intestinal polyposis syndromes and hereditary colorectal cancer" and "colorectal neoplasia", perhaps by necessity, stray somewhat from the clinical emphasis to include a more

detailed account of the pathogenesis and epidemiology. They cover the basic genetic aspects in rather more detail than is the trend in other chapters and offer more discussion of the evidence base rather than being confined to expert interpretation and opinion. This perhaps deflects from the needs of the intended target audience towards those more familiar with the subject. Unlike the other chapters, there is also a degree of overlap between these two chapters, which is particularly related to the clinical and genetic criteria for diagnosis of HNPCC and the polyposis syndromes, and the criteria for genetic testing and screening. Both chapters are well written and interesting but it might have been more in keeping with the aims of the book to have combined them and kept to a more clinical approach. The subject matter of the text is augmented by key points in boxes which summarise areas of particular importance covered in each chapter, thus providing a useful at a glance reference. Several chapters contain investigation and treatment algorithms. These are particularly useful in the chapters on diarrhoea, inflammatory bowel disease, and colorectal neoplasia, and would have been a welcomed addition to other areas, particularly the investigation of malabsorption.

There is a strong sense that this text has been prepared with certification and recertification of North American Physicians in mind but it is also well suited to medical students in the broader sense, perhaps revising for finals, MRCP candidates, and medical registrars in training. Although not referenced, each chapter offers a guide to further reading providing a useful introduction to the scientific literature. In this volume, editors Lichtenstein and Wu achieve the aims of the series, as indicated by Anil Rustgi, the editor in chief in his foreword, to provide a user friendly text, imparted with expert knowledge and insights, that together constitute an overview and refresher course aimed at those training in the field of gastroenterology and those in other areas of medicine who want a succinct pragmatic overview.

S J Middleton

## CORRECTION

doi: 10.1136/gut.2003.035113corr1

The spelling of one of the authors in the paper by Byrne *et al* (CD4<sup>+</sup>CD45RB<sup>HI</sup> T cell transfer induced colitis in mice is accompanied by osteopenia which is treatable with recombinant human osteoprotegerin, 2005;**54**:78–86), published in the January 2005 issue, was incorrect. R Manuokian should read R Manoukian.