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 aetiology 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 though recent data have questioned the functional effect of this variant.

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. Tokor et al examined the presence of both the Asp299Gly and Thr399Ile polymorphisms in a smaller German IBD population. 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.

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 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.

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 Ghananian controls (carrierage 1%). 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 and Finland and are absent in a small Icelandic population.

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.

<table>
<thead>
<tr>
<th>Patient numbers (allele frequencies)</th>
<th>CD</th>
<th>UC</th>
<th>HC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leuven</td>
<td>334</td>
<td>163</td>
<td>139</td>
</tr>
<tr>
<td>Munich</td>
<td>102</td>
<td>98</td>
<td>145</td>
</tr>
<tr>
<td>Edinburgh</td>
<td>234</td>
<td>246</td>
<td>189</td>
</tr>
</tbody>
</table>

HC allele frequencies differed between Edinburgh and Munich (p<0.02, odds ratio 1.35 (confidence interval 1.1–4.51) and those between Edinburgh and Leuven approached significance (p=0.05).

References

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 proposing 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...
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. Complications are rare, the main one being that it can become stuck with no apparent manoeuvre or fluid ingestion. The incidence in published series range from 1% to 5%, and all were managed with a surgical or endoscopic procedure. 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 64 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 auralic fibrillation. She had undergone upper and lower gastrointestinal endoscopy and mesenteric angiography but capsule passed delayed stenosis 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 was stuck at a pulsate area in the distal oesophagus, staying there for up to three hours and passing afterwards without any specific related cause (fig 2). The study
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).

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.1 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.2 Infected cirrhotics demonstrate a heparin effect using heparinase I modified thromboelastography (TEG) and have anti-Xa activity.3 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.7 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:8 a heparin effect was defined as an improvement in r time, k time, and ζ 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 “ζ angle” (the slope drawn...
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 over time: in patient 1, \( r \) time 11 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. 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 linked to 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.4

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doi: 10.1136/gut.2004.051474
Conflict of interest: None declared.

References

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.1 After this condition was found to be associated with malabsorption as well as the presence of alpha heavy chain proteins,2 the disease was named by the WHO as “immunoproliferative small intestinal disease” (IPSID).3 One of the earliest reports of IPSID was from our centre.4 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 two 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.

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 comprised 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 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 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.1 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.5 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.6 Vaccination programmes increased dramatically after the Islamic revolution, reaching more than 90% of children.7 Local health facilities increased dramatically during the first two decades after the revolution.

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

<table>
<thead>
<tr>
<th>Age group (y)</th>
<th>Total</th>
<th>ASR Males</th>
<th>ASR Females</th>
<th>ASR</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–4</td>
<td>2</td>
<td>0.02</td>
<td>1</td>
<td>0.02</td>
</tr>
<tr>
<td>5–9</td>
<td>6</td>
<td>0.05</td>
<td>6</td>
<td>0.1</td>
</tr>
<tr>
<td>10–14</td>
<td>5</td>
<td>0.05</td>
<td>4</td>
<td>0.08</td>
</tr>
<tr>
<td>15–19</td>
<td>14</td>
<td>0.17</td>
<td>5</td>
<td>0.12</td>
</tr>
<tr>
<td>20–24</td>
<td>35</td>
<td>0.54</td>
<td>14</td>
<td>0.43</td>
</tr>
<tr>
<td>25–29</td>
<td>35</td>
<td>0.59</td>
<td>17</td>
<td>0.62</td>
</tr>
<tr>
<td>30–34</td>
<td>11</td>
<td>0.25</td>
<td>7</td>
<td>0.31</td>
</tr>
<tr>
<td>35–39</td>
<td>16</td>
<td>0.43</td>
<td>11</td>
<td>0.38</td>
</tr>
<tr>
<td>40–44</td>
<td>1</td>
<td>0.38</td>
<td>1</td>
<td>0.34</td>
</tr>
<tr>
<td>45–49</td>
<td>10</td>
<td>0.42</td>
<td>7</td>
<td>0.57</td>
</tr>
<tr>
<td>50–54</td>
<td>7</td>
<td>0.32</td>
<td>7</td>
<td>0.61</td>
</tr>
<tr>
<td>55–59</td>
<td>5</td>
<td>0.28</td>
<td>3</td>
<td>0.31</td>
</tr>
<tr>
<td>60–64</td>
<td>2</td>
<td>0.12</td>
<td>2</td>
<td>0.22</td>
</tr>
<tr>
<td>&gt;65</td>
<td>2</td>
<td>0.13</td>
<td>2</td>
<td>0.25</td>
</tr>
<tr>
<td>Total</td>
<td>161</td>
<td>0.21</td>
<td>98</td>
<td>0.25</td>
</tr>
</tbody>
</table>

Age specific rate per 100 000 population in each age group.
We postulate that improvement in health in general and decreasing childhood gastrointestinal enteropathies 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.

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Conflict of interest: None declared.

Microscopic (collagenous and lymphocytic) colitis triggered by food allergy

Collagenous and lymphocytic colitis are rare diseases of unknown aetiology but several issues,1, 2 in particular the good response to budesonide,3 are suggestive of immunopathology. Patients have watery diarrhoea without abnormal findings on colonoscopy4 but with increased numbers of intraepithelial lymphocytes, mast cells, and eosinophils5 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 measured6 on a normal and hypoallergenic potato–rice–diet. Colonoscopy with endoscopically guided segmental lavage for intestinal IgE was carried out7 and biopsies were investigated by routine pathology (haematoxylin–eosin), immunohistochemistry for eosinophil peroxidase, and amount of eosinophil cationic protein and tryptase9 in the whole biopsy. Clinical activity was mainly assessed by number of stools/day 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, eosinophil 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. Allergens could induce increased eosinophil infiltration and enhance transforming growth factor β2 with increased collagen deposition. Eosinophils are highly susceptible to steroids which may explain the good response of collagenous colitis to budesonide.4

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.

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doi: 10.1136/gut.2004.049767

Table 2 Number of stools per day (Baets’ score) before and after therapy

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age (y)</th>
<th>Follow up (y)</th>
<th>Atopy</th>
<th>Signs of allergy</th>
<th>Diagnostic markers suggestive of allergy</th>
<th>Identified allergens</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>49</td>
<td>5</td>
<td></td>
<td>Improvement by hypoallergenic diet</td>
<td>Eos in bx</td>
<td>Egg, pollen, house dust mite</td>
</tr>
<tr>
<td>M</td>
<td>45</td>
<td>5</td>
<td></td>
<td>Urticaria, improvement by cromolyn</td>
<td>Eos in bx</td>
<td>Spices, moulds, flours, celery, nuts, milk, maize, rice, apple, celery, soy</td>
</tr>
<tr>
<td>M</td>
<td>46</td>
<td>5</td>
<td></td>
<td>Pansusitis</td>
<td>Eos in bx</td>
<td>Maize starch</td>
</tr>
<tr>
<td>M</td>
<td>52</td>
<td>3</td>
<td></td>
<td>IgG deficiency</td>
<td>Eos in bx</td>
<td>Milk, egg, soy</td>
</tr>
<tr>
<td>M</td>
<td>71</td>
<td>9</td>
<td></td>
<td>Prick</td>
<td>Prick, RAST</td>
<td>Ananas</td>
</tr>
<tr>
<td>F</td>
<td>59</td>
<td>4</td>
<td></td>
<td>Prick</td>
<td>UMH</td>
<td></td>
</tr>
</tbody>
</table>

Eos in bx, eosinophils in colon biopsy; UMH, urine methylhistamine.
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 dysplastic mucosa. Hata et al. pointed out, a reference to this article by Hata et al. (Gut 2000;49:1198–9). Weidenhiller M, and Schiller LR. Pathophysiology and treatment of microscopically diagnosed colitis. Lancet 2000;355:1198–9.


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!”

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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 fibrinous 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. 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. 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 α (TNF-α) in this condition, TNF-α mRNA was measured in the
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-α 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 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. The pathogenesis of cap polyposis remains unclear. Our data do not support the hypothesis that TNF-α 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.

It has therefore been suggested that abnormal colonic motility may be an important aetiological factor.

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doi: 10.1136/gut.2004.053686
Conflict of interest: None declared.

References

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 α (TNF-α) therapies in Crohn’s disease (CD) may critically depend on the binding of the transmembrane precursor of TNF-α (mTNF-α), thus eliciting complex intracellular signalling events, a process described as “reverse signalling”. It has also been suggested that failure of another TNF binding agent, etanercept (Enbrel; a recombinant TNFRII:Fc fusion protein), to induce peripheral and lamina propria lymphocyte apoptosis, provides a possible molecular explanation for the lack of efficacy of etanercept in a randomised placebo controlled trial in active CD.

Figure 1

Intestinal CD4+ 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-α treatment. Neither antigen nor feeder cells were added to the intestinal T cells cultures, which consisted of more than 97% CD3+/CD4+ T cells. (A) Infliximab, but not etanercept, activated p38α 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-α binding agent, as described previously. Data are representative of experiments performed in all patients, n = 5. (B) Signal transducer and activator of transcription 3 (STAT3) was activated in CD4+ 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. 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-α by infliximab (for example, they 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). 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 TNF-α. 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.  

According to the findings of van den Brande et al., we observed PARP cleavage as a molecular hallmark of apoptosis in cultures grown with infliximab (fig 1A) 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 p38, 24 hours after infliximab treatment was observed in 4/5 of the cell lines derived from CD patients (fig 1A)).

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. Tyrosine phosphorylated STAT3 can be found in CD4 T cells. The reason for this discrepancy probably lies in the complex and dichotomal 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, and it shares differential actions in different T cell subpopulations. In naïve T cells, p38 can drive proliferation and cytokine production in both Th1 and Th2 cytokines to the former. Since several lines of evidence have also suggested the proapoptotic role of the p38 pathway, Waetzig and colleagues 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 as well as the inability of infliximab to neutralise downstream of p38MAPK are not related to infliximab induced immune cell apoptosis.

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, STAT1 and STAT3 proteins are constitutively activated in lamina propria CD4+ T cells from active Crohn’s disease patients. The finding of Rosenstiel et al that both infliximab and etanercept are able to reduce in vitro STAT3 tyrosine phosphorylation in T cells from active Crohn’s disease patients is probably related to the capacity of both of these agents to neutralise soluble TNF-α. Interestingly, the absence of quantitative differences in the differential p53 increase in p38 and etanercept in downregulating STAT3 signalling is consistent with the data of van den Brande and colleagues who have shown that both drugs can neutralise soluble TNF-α 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-α 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...
Small and Large Intestine


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 entrenched 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 and in 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 more discussion 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.

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CORRECTION

doi: 10.1136/gut.2003.05113corr1

The spelling of one of the authors in the paper by Byrne et al (CD4+CD45RBHi 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 Manoukian should read R Manoukian.