Figure 1 Interleukin (IL) 15 production after gluten challenge. Representative IL15 western blot of (A) whole biopsy protein extract of a responding patient with coeliac disease (CD) on gluten-free diet (GFD) challenged with gliadin compared with basal culture, and two patients without CD challenged with gliadin and the synthetic gliadin peptides 19-mer and deaminated 33-mer, respectively, compared with the basal culture. Interestingly, none of the basal cultures produced this cytokine. (B) 24 h culture supernatant (SN) of a patient without CD basal culture and the 3 and 24 h culture supernatants of the same patient after challenge with the 19-mer and deaminated 33-mer, respectively. IL15, positive control lane.

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SR and LF-S are the gastroenterologists who performed the follow-up of the patients as well as provided the duodenal biopsy samples. DB performed the biopsy cultures and the western blot analysis, and JAG performed the qPCR and statistical analyses. The study design was carried out by EA.

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The value of endothelin 1 in the early diagnosis of severe intestinal strangulation

In patients with intestinal strangulation, early diagnosis and prompt intervention can play a

key role in the final outcome. Physical signs and routine laboratory findings only raise the level of suspicion to the presence of intestinal ischaemia. Several markers such as alkaline phosphatase,¹ creatinine phosphokinase,² and lactate dehydrogenase³ and procalcitonin (Pct) serum levels have been proposed for the early diagnosis of intestinal ischaemia.⁴

Plasma levels of endothelin 1 (ET1) have been recently associated with cardiac ischaemia.⁵ It has also been reported that ET1 plays a central role in the pathophysiology of intestinal ischaemic injury.⁶ Wang *et al*⁷ recently reported that ligation of mesenteric vessels caused an increase in serum ET1 levels in rats. On the other hand, other researchers reported that in spite of increased release of ET1 from the strangulated loop, no statistically significant increase of ET1 in peripheral blood samples can be established.⁸ These controversial results may be attributed to the occlusion of the local venous vessels.

In an attempt to further study these controversial data, we performed concomitant ligation of the arterial and venous vessels that correspond to the occluded loop. It is also noted that this approach may better reflect the sequence of events that follow the onset of severe strangulation.

In 11 New Zealand rabbits, the intestinal lumen and the corresponding mesenteric vessels were occluded using silk sutures, one at 30 cm from the duodenum and one 15 cm distal to the first ligation. This model reproduces the phenomenon of the obstruction of intestinal wall perfusion due to severe strangulation and the pathophysiological processes at the onset of ischaemia that complicates the natural history of the disease. For the six controls, the steps of the operation were exactly the same, except the strangulation of the jejunum was not performed.

Blood samples were drawn preoperatively and consequently at 30, 60, 180 and 360 min after strangulation. At the same time intervals, a transmural biopsy specimen was obtained from the strangulated loop. Tissue samples were embedded in paraffin wax and submitted for H&E staining. Ischaemic injury of the intestine was scaled in four degrees of severity.⁹

The plasma concentration of ET1 was estimated with a commercial immunoassay kit (Assay Designs, Ann Arbor, Michigan, USA).

The comparison of ET1 values between the two groups revealed higher concentrations in the ischaemia group, at every time interval, which were statistically significant for all postoperative samples ($p < 0.05$; fig 1). ET1 levels in plasma increased as early as 30 min after intestinal strangulation, and continued to increase with time up to 10-fold in the next 360 min. By contrast, in the control group no statistically significant alteration of ET1 levels, within the measured samples, was observed.

According to the analysis of histopathological grading of ischaemic injury, the ischaemia group samples were graded as follows: at 30 min, 2 samples of grade I; at 60 min, 8 of grade II and 2 of grade I; at 180 min, 3 of grade II and 8 of grade III; and, finally, at 360 min 1 of grade I and 10 of grade IV. No evidence of ischaemic injury was detected in specimens derived from the control group. ET1 plasma levels correlated with histopathological damage. Mild increase in ET1 levels reflected mild, mainly mucosal intestinal ischaemic injury graded in this study as grade I and II, whereas a twofold or higher increase in circulating ET1 is consistent with extensive epithelial injury affecting the mucosa and the submucosa, and the muscle layers of the intestinal wall classified as grade III and IV.

It is evident that plasma levels ET1 rise at 30 min of strangulation before any histological

Figure 1 Variation of endothelin 1 levels in peripheral blood samples (* $p < 0.05$).

damage is observed. This makes ET1 an additional useful marker for severe intestinal strangulation than other hitherto reported markers including procalcitonin.⁴ According to these results, measurement of plasma ET1 in a clinical setting is justified to further evaluate its value in the early diagnosis of intestinal ischaemia due to strangulation.

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The RANTES -28 g polymorphism is associated with primary sclerosing cholangitis

Primary sclerosing cholangitis (PSC) is a chronic cholestatic disorder commonly associated with inflammatory bowel disease (IBD). The pathogenesis of PSC remains unknown, but bacterial translocation from the gut to the portal circulation, resulting in activation of an immune cascade and subsequent bile duct injury, is believed to be one of the triggering factors. In a previous study,¹ we found a significantly lower frequency of the Δ32 deletion in the CC-type chemokine receptor 5 (CCR5) gene in patients

Figure 1 The RANTES (*Regulated on Activation Normal T Expressed and Secreted*) gene and the position of the different studied polymorphisms.

with PSC compared with patients with IBD and healthy controls (HC), suggesting a protective effect of this mutation on the development of PSC. Given this association in our population, we further investigated the role of RANTES (*Regulated on Activation Normal T Expressed and Secreted*, also known as CC-motif chemokine ligand 5 (CCL5)), one of the ligands for the CCR5 receptor.

RANTES, localised to the cytokine cluster on chromosome 17q11.2-q12, is a plausible candidate gene for PSC, given its proinflammatory properties both independent of and as a ligand for the CCR5 receptor. A higher expression of RANTES has been reported in the gut epithelium and lymphocytes of patients with IBD.² Moreover, in an animal model of colitis, treatment with RANTES antagonists resulted in decreased bacterial translocation, as measured by portal blood endotoxin levels.³

Two promoter polymorphisms in RANTES (-28G and -403A) result in increased transcription of the gene, whereas a single-nucleotide polymorphism (SNP) in the first intron (In1.1C) results in reduced transcription of RANTES.⁴ We studied these three SNPs together with a fourth known SNP of as yet unknown function in the 3'UTR (3'222), for their role in susceptibility to PSC, and behaviour and progression of PSC (fig 1).

We genotyped 124 patients with PSC (63 without IBD, 25 with Crohn's disease (CD), 31 with ulcerative colitis (UC) and 5 with indeterminate colitis (IC)) for the -403G/A, -28C/G, In1.1T/C and 3'222T/C variants in the RANTES gene. Patients with PSC and IBD were recruited from the hepatology and gastroenterology departments of the same tertiary care referral centre. Table 1 summarises and compares the disease characteristics of IBD in patients with and without PSC. Genotype and

allele frequencies were compared with 595 patients with IBD without PSC (418 CD, 160 UC, 17 IC), and with 362 HC. SPSS V.14.0 software was used for statistical analysis, Haploview for calculation of linkage disequilibrium (LD)⁵ and fastPHASE 1.1.4 for haplotype estimation.⁶

Allele frequencies for the studied polymorphisms are summarised in table 2. All studied polymorphisms were in Hardy-Weinberg equilibrium. The -28G allele was significantly more frequent in patients with PSC (5.9%) compared with those with IBD (3.4%, $p=0.05$) and HC (2.2%, $p=0.005$). Furthermore, in patients with CD, the -28G allele was significantly more frequent in those patients who developed PSC (10.4%) compared with those who did not develop PSC (3%, $p=0.005$), with an odds ratio (OR) of 4.16 (95% CI 1.4 to 12). The allele frequency for the RANTES 3'222 allele was <1% in our cohort. The -403, -28 and In1.1 SNPs were all in strong LD (D' 0.838 for -403/-28, 0.946 for -403/In1.1 and 0.78 for -28/In1.1). Haplotype analysis could not identify differences between the studied groups. There was no association between any of the studied RANTES SNPs and location and behaviour of IBD or PSC in the different subgroups, nor with the CCR5Δ32 mutation.

In conclusion, this is the first report of an association between a functional promoter polymorphism in the RANTES gene, with an OR of 4.16 for the development of PSC in patients with CD. This -28G promoter polymorphism is known to result in increased RANTES transcription and expression. Given its known proinflammatory properties, and taking the data on the action of RANTES antagonists in experimental colitis into account,³ a possible mechanism to explain the

Table 1 Characteristics of patients with IBD and of patients with PSC with concomitant IBD

	Crohn's disease		Ulcerative colitis	
	CD with PSC n=25 (%)	CD without PSC n=418 (%)	UC with PSC n=31 (%)	UC without PSC n=160 (%)
Localisation of CD				
Ileal	1/25 (4)	107/396 (27)	—	—
Colonic	8/25 (32)	65/396 (16)	—	—
Ileocolonic	15/25 (60)	177/396 (45)	—	—
Upper	1/25 (4)	47/396 (12)	—	—
Anal involvement	8/25 (35)	132/392 (34)	—	—
Behaviour of CD				
Inflammatory	16/25 (64)	144/386 (37)	—	—
Strictureing	3/25 (12)	86/386 (22)	—	—
Penetrating	6/25 (24)	156/386 (40)	—	—
Localisation of UC				
Proctitis	—	—	3/31 (10)	33/146 (23)
Left-sided colitis	—	—	10/31 (32)	66/146 (45)
Extensive colitis	—	—	18/31 (58)	47/146 (32)

CD, Crohn's disease; PSC, primary sclerosing cholangitis; UC, ulcerative colitis.