be room for improvement by raising the dose. The findings merit clinical development of anti-CD-WPC, and should be confirmed in a prospective placebo-controlled randomised trial.

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Is gliadin really safe for non-coeliac individuals? Production of interleukin 15 in biopsy culture from non-coeliac individuals challenged with gliadin peptides

Nowadays it is assumed that an innate immunity to gluten plays a key role in the development of coeliac disease (CD).[1] This innate response, mediated by interleukin (IL) 15 and elicited by ''toxic peptides'', like the 19-mer, through a DQ2-independent mechanism, induces epithelial stress and reprogrammes intraepithelial lymphocytes into natural killer (NK)-like cells[2] leading to enterocyte apoptosis and an increase in epithelium permeability. Thus, immunodominant peptides, like the 33-mer, can reach the lamina propria to trigger adaptive immunity. However, although an innate specific response in CD has been reported,[3] no differential factors between patients with and without CD have been described controlling the innate immune response. Thus, since the toxic 19-mer elicits its harmful effect through a DQ2-independent mechanism, we hypothesise that the innate response is common in patients with and without CD, whereas the adaptive response is exclusive of susceptible patients with CD.

Table 1 Gliadin-challenged patients without coeliac disease

Patient no	Duodenal mucosa	Age (years)	Symptoms	Diagnosis
1	Normal	51	Unfiliated ferropenia	Undiagnosed (non-coeliac)
2	Normal	63	Pyrosis	Hiatus hernia, GORD
3	Chronic inflammation	46	Colic abdominal pain	Chronic gastritis
4	Chronic inflammation	54	Epigastric pain	Chronic gastritis
5	Normal	16	Diarrhoea	Protracted diarrhoea
6	Normal	71	Dyspepsia	Polyps in stomach, Helicobacter

GORD, gastroesophageal reflux disease.
The final diagnosis of the patients was non-coeliac in all the cases. Those with chronic inflammation had normal villi as well as no intraepithelial lymphocytosis. All the patients were biopsy cultured in basal medium and challenged with both the 19- and the 33-mer gliadin peptides. Patients 1–3 were also challenged with gliadin.

To test the hypothesis, biopsy cultures were taken from at least three patients with CD who are on a gluten-free diet (GFD) and three patients without CD (table 1). Biopsy specimens were challenged with crude gliadin and the gliadin synthetic 19-mer and deaminated 33-mer peptides after discarding the presence of lipopolysaccharide in all the cases. This was carried out at 100 μg/ml for only 3 h to imitate what are considered the normal timing and concentration in the gut after a normal meal. All biopsy specimens were then washed and cultured for another 21 h in new clean culture medium to determine whether an innate stimulus is reflected by an adaptive response. Each sample cultured in basal medium constituted an internal control. Innate immune mediators IL15 and nitrites were determined by western blot in the biopsy protein extract and by a Griess reagent system in the 3 h supernatants respectively. mRNA levels of adaptive immunity mediators like signal transducers and activators of transcription (STAT) 1, STAT3, tumour necrosis factor α, interferon (IFN) γ, IL23 (p19), IL27 (p28) and IL12 (p35) were determined by real-time polymerase chain reaction using β actine levels as housekeeping.

All patients with and without CD on GFD who were challenged with the gliadin solution produced IL15 when compared with the basal culture (fig 1A). Moreover, the IL15-mediated response in patients without CD was also triggered by the toxic 19-mer gliadin peptide (three of six) and, especially, by the 33-mer gliadin peptide (five of six). Importantly, none of the basal cultures produced this cytokine and, although not expected, the ''non-toxic'' immunodominant 33-mer was also able to induce an innate response. Interestingly, this IL15 response was also confirmed by western blot in the supernantant of one GFD patient with CD and three patients without CD, who were on GFD (fig 1B), therefore, discarding an intracellular and non-biologically active IL15 response in patients without CD. We also found an increase in nitrite in the gliadin-challenged patients with CD who were on a GFD, although not in patients without CD. In a similar way, as expected, after the biopsy mRNA isolation, adaptive mediators (STAT1, STAT3, IFNγ) were only modified in GFD patients with CD. Finally, basal GFD-CD samples showed an 80-fold increase in IFNγ mRNA levels compared with non-CD basal samples (p value 0.002) and a slightly higher production of nitrites (p value 0.052).

We consider that, to our knowledge, this is the first time that an IL15-mediated innate response to gliadin and gliadin peptides is described in individuals without CD, as well as an IL15-mediated innate response to the ''non-toxic'' deaminated immunodominant 33-mer peptide.

The data obtained in this pilot study support the hypothesis that gluten elicits its harmful effect, throughout an IL15 innate immune response, on all the individuals. This innate response is found in both patients with and without CD, although the triggering of an adaptive response is CD specific. We propose that somehow patients with CD need to be DQ2 and also have a lower threshold for triggering an adaptive TH1 response. This lower threshold could be mediated by the higher basal levels of immune mediators, like IFNγ mRNA, found in patients with CD, a defect in the CD permeability or even a higher IL15-sensitive response under the same stimulus, which might be mediated by a higher density of IL15 receptor in patients with CD.[4]

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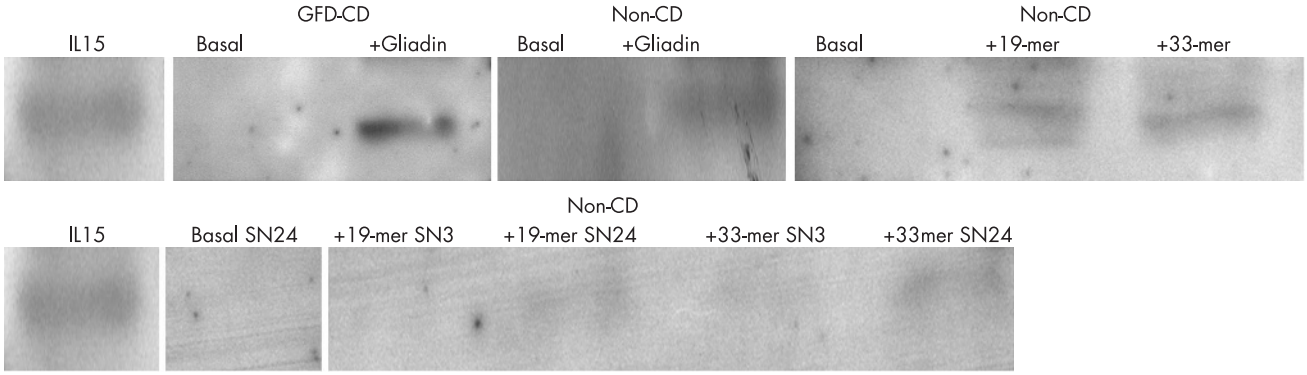

Figure 1 Interleukin (IL) 15 production after gluten challenge. Representative IL15 western blot of (A) whole biopsy protein extract of a responding patient with coeliac disease (CD) on gluten-free diet (GFD) challenged with gliadin compared with basal culture, and two patients without CD challenged 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 phosphtase,[1] creatinine phosphokinase,[2] and lactate dehydrogenase[3] 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

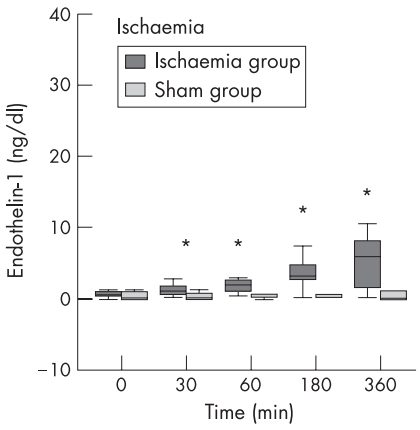

Figure 1 Variation of endothelin 1 levels in peripheral blood samples (*$p<0.05$).