

# Histological changes associated with wheat protein antibodies in the absence of villous atrophy

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**SUMMARY** A retrospective study was conducted to assess the association of  $\alpha$ -gliadin antibodies with intraepithelial lymphocyte counts. Twelve subjects with apparently normal small intestinal histology and raised  $\alpha$ -gliadin antibody titres had significantly increased intraepithelial lymphocyte counts (42 (SEM) 5.9) when compared with 16 subjects with normal  $\alpha$ -gliadin antibody titres (17 (3.2);  $p < 0.001$ ). These findings show that in the absence of gross pathology raised  $\alpha$ -gliadin antibody titres are associated with increased numbers of intraepithelial lymphocytes and may reflect continuous immunological processes in the small intestine.

Raised intraepithelial lymphocyte (IEL) counts are characteristic of the coeliac small intestinal lesion.<sup>1,2</sup> The numbers decrease if the patient maintains a strict gluten free diet, although values generally remain abnormal.<sup>3</sup> Raised titres of  $\alpha$ -gliadin antibodies (AGAs), which decrease with treatment, are also associated with coeliac disease<sup>4</sup> and are found in 90% of newly diagnosed adult patients<sup>5</sup> and 95% of children with coeliac disease.<sup>6</sup>

As part of a continuing investigation into coeliac disease in this centre small intestinal biopsy specimens and AGA titres are examined routinely in patients with possible malabsorption. A proportion of these subjects (15%) have raised AGAs, although their small intestinal histology appears to be normal. This retrospective study investigates whether there is an association between raised AGA titres and increased IEL counts in these subjects.

## Patients and methods

Fifty three subjects were studied retrospectively. There were two distinct groups. In 25 patients with abnormal small intestinal histology, coeliac disease was diagnosed using established criteria; and a gluten free diet was prescribed. Ten of these patients were positive for AGAs and 15 were negative for AGAs at

the time of diagnosis. The remaining 28 patients had normal small intestinal histology and the diagnosis of coeliac disease was excluded; 16 were negative for AGAs and 12 were positive for AGAs. The table lists the clinical features of these 28 patients.

AGAs were estimated using the enzyme linked immunosorbent assay (ELISA) described previously.<sup>4</sup> An ELISA index greater than two standard deviations above the mean of a group of 30 healthy subjects was said to be positive.

During the initial investigation, routine histological analysis was carried out on each biopsy specimen. Subsequently, as part of the retrospective study, the lymphocytic infiltrate between surface epithelial cells (intraepithelial lymphocytes) was assessed using the technique previously described by Ferguson *et al.*<sup>1</sup>

Table Clinical features of 28 patients in whom diagnosis of coeliac disease was excluded because small intestinal histology appeared to be normal

<b>Alpha gliadin antibody positive (n = 12)</b>	
Iron deficiency anaemia	4
Diarrhoea; weight loss	4
Dermatitis herpetiformis	1
Recurrent oral ulceration	3
<b>Alpha gliadin antibody negative (n = 16)</b>	
Healthy volunteers	5
Iron deficiency anaemia	1
Transient diarrhoea and weight loss	4
Irritable bowel syndrome	4
Recurrent oral ulceration	2

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Between 500 and 1000 epithelial cells were counted and the number of lymphocytes present was expressed as a percentage. This method is currently accepted as suitable for quantifying IEL counts in patients being investigated for coeliac disease.<sup>7</sup>

Because the data were not normally distributed, the Mann-Whitney U test was used to assess the significance of the differences in IEL counts between the two groups.

### Results

Using standard microscopy, all 25 patients with coeliac disease showed the typical histological pattern of villous atrophy, crypt hyperplasia, enterocyte disarray and inflammatory infiltrate. The histology of the small intestine of the remaining 28 patients appeared to be normal with normal villi, surface epithelium, and cellular infiltrate.

On retrospective analysis of patients with apparently normal small intestinal histology, IEL counts were increased in those positive for AGAs (42.4 (5.9)) when compared with those negative for AGAs (17 (3.2)). This difference was highly significant  $p < 0.001$ . Increased IEL counts were also seen in both groups of patients with coeliac disease. Although the count was higher in those positive for AGAs (82.5 (9.5)) when compared with those negative for AGAs (74.3 (5.6)), the difference was not significant (figure).

### Discussion

This study shows that increased intraepithelial lymphocyte (IEL) counts are found in patients with otherwise normal small intestinal histology who have raised titres of  $\alpha$ -gliadin antibodies (AGAs). The importance of this finding is unknown, although the association of an enhanced humoral response to a dietary antigen and an increase in one population of gastrointestinal immunocompetent cells suggests that the mucosal immune system is activated. These features may not be associated with any specific pathogenic process but may simply characterise those subjects with a pronounced gastrointestinal immune response.

Raised titres of wheat protein antibodies and increased IEL counts that decrease on maintenance of a gluten free diet are commonly associated with coeliac disease.<sup>8,9</sup> Gluten sensitive disease, however, has also been described when the only small intestinal histological abnormality was increased IEL counts. Dermatitis herpetiformis is a gluten sensitive skin disease that is sometimes associated with villous atrophy.<sup>10</sup> Patients with this disease whose small intestinal histology appears to be normal have

increased IEL counts<sup>11,12</sup> which decrease after treatment with a gluten free diet.<sup>14</sup> In another study eight patients with gluten sensitive diarrhoea and normal small intestinal histology had increased IEL counts that returned to normal with a gluten free diet.<sup>14</sup> Increased IEL counts and raised AGA titres may therefore be useful indicators of wheat protein sensitive disease in the absence of gross small intestinal abnormality.

The precise role of IELs in the normal intestine is unknown,<sup>7</sup> although these cells have been shown to be of suppressor phenotype<sup>15</sup> and so may have an immunoregulatory role. Studies on animals have indicated that raised IEL counts are associated with a local cell mediated immune response in the intestine.<sup>16,17</sup> It has been suggested that this response is the first in a chain of events which can lead to the generation of mucosal damage.<sup>18</sup> Increased numbers of IELs in untreated wheat protein sensitive disease, together with the decrease seen on removal of gluten from the diet and the increase on gluten challenge,<sup>19,20</sup> suggest that IELs participate in a gluten specific immune response which may be pathogenic.

It has been argued that the reduction in epithelial volume, associated with the small intestinal damage

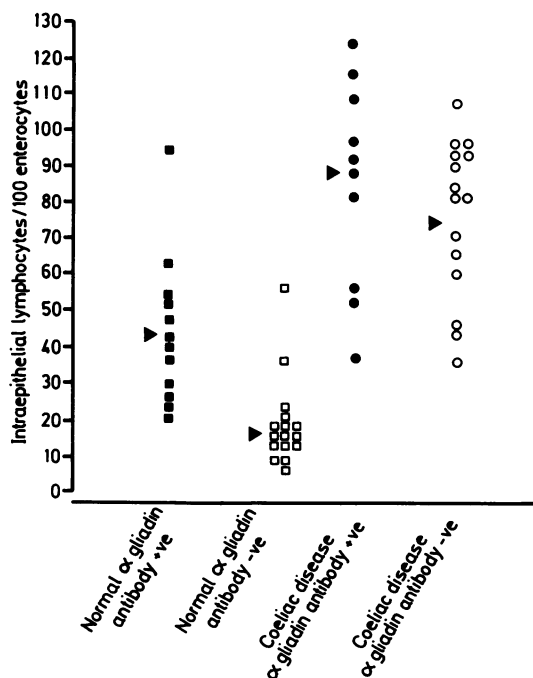


Figure Intraepithelial lymphocyte (IEL) counts in alpha gliadin antibody (AGA) positive and negative coeliac patients and AGA positive and negative patients with normal small intestinal histology.

characteristic of untreated coeliac disease, causes an increase in IEL density but not necessarily in cell numbers.<sup>21</sup> In our study patients with coeliac disease had high IEL counts, irrespective of whether they had raised or normal AGA titres. The mechanical effect of villous atrophy on IEL density probably masks the more subtle influence of a local gluten sensitive immune response on IEL infiltration. In a study of patients with treated coeliac disease whose small intestinal mucosa had returned to normal a dose dependent incremental rise in absolute numbers of IELs occurred on challenge with varied small doses of gluten before any deterioration in gross mucosal architecture.<sup>22</sup> Thus it seems likely that a local immune response to wheat protein indicated by IEL infiltration and raised AGA titres may precede a more severe manifestation of gluten sensitive disease.

In conclusion, we have shown that patients whose small intestinal mucosa appears to be normal but whose AGA titres are raised have increased numbers of IELs. The association of increased numbers of circulating wheat protein antibodies with increased numbers of IELs may reflect an active immunological process, which is related to the presence of gluten in the small bowel.

#### References

- 1 Ferguson A, Murray D. Quantitation of intraepithelial lymphocytes in human jejunum. *Gut* 1971;12:988-94.
- 2 Ferguson A. Intraepithelial lymphocytes of the small intestine: Progress report. *Gut* 1977;18:921-37.
- 3 Holmes GKT, Asquith P, Stokes PL, Cooke WT. Cellular infiltrate of jejunal biopsies in adult coeliac disease in relation to gluten withdrawal. *Gut* 1974;15:278-83.
- 4 O'Farrelly C, Kelly J, Hekkens WIJM, *et al*. Alpha gliadin antibody levels: a serological test for coeliac disease. *Br Med J* 1983;286:2007-10.
- 5 O'Farrelly C, Feighery C, O'Briain S, *et al*. Humoral response to wheat protein in coeliac disease and enteropathy associated T cell lymphoma. *Br Med J* 1986;293:908-10.
- 6 Kelly J, O'Farrelly C, O'Mahony C, *et al*. Alpha gliadin antibodies in childhood coeliac disease—a screening test. *Gut* 1984;25:A548.
- 7 Dobbins WO. Human intestinal intraepithelial lymphocytes. *Gut* 1986;27:972-85.
- 8 Strober W. Gluten sensitive enteropathy—an abnormal immunologic response of the gastrointestinal tract to a dietary

protein. In: *Gastrointestinal immunity for the clinician*. Orlando, Florida: Grune & Stratton, 1985:75-112.

- 9 Mike N, Asquith P. Gluten toxicity in coeliac disease and its role in other gastrointestinal disorders. In: Brostoff J, Challacombe S, eds. *Food allergy and intolerance*. London: Ballière Tindall, 1987:521-48.
- 10 Fry L, McMinn RMH, Cowan JD, Hoffbrand AV. Effect of gluten-free diet on dermatological, intestinal and haematological manifestations of dermatitis herpetiformis. *Lancet* 1968;i:557-61.
- 11 Fry L, Seah PP, McMinn RMH, Hoffbrand AV. Lymphocytic infiltration of epithelium in diagnosis of gluten sensitive enteropathy. *Br Med J* 1972;3:371-4.
- 12 Fry L, Seah PP, Harper PG, Hoffbrand AV, McMinn RMH. The small intestine in dermatitis herpetiformis. *J Clin Pathol* 1974;27:817-24.
- 13 Kumar PJ, Silk DBA, Marks R, Clark ML, Dawson AM. Treatment of dermatitis herpetiformis with corticosteroids and a gluten-free diet: a study of jejunal morphology and function. *Gut* 1973;14:280-3.
- 14 Cooper BT, Holmes GKT, Ferguson R, Thompson RA, Allen RN, Cooke WT. Gluten-sensitive diarrhea without evidence of celiac disease. *Gastroenterology* 1980;79:801-6.
- 15 Selby WS, Janossy G, Jewell DP. Immunohistological characterisation of intraepithelial lymphocytes of the human gastrointestinal tract. *Gut* 1981;22:169-76.
- 16 Guy-Grand D, Griscelli C, Vasalli P. The mouse gut T lymphocyte, a novel type of T cell: nature, origin and traffic in mice in normal and graft versus host conditions. *J Exp Med* 1978;148:1661-77.
- 17 Mowat A, Ferguson A. Hypersensitivity in the small intestine. V Induction of cell mediated immunity to a dietary antigen. *Clin Exp Immunol* 1981;43:574-82.
- 18 Strobel S, Shields JG. The mucosal T cell. In: Brostoff J, Challacombe S, eds. *Food allergy and intolerance*. London: Ballière Tindall, 1987:103-17.
- 19 Lancaster-Smith M, Kumar PJ, Dawson AM. The cellular infiltrate of the jejunum in adult coeliac disease and dermatitis herpetiformis following the reintroduction of gluten. *Gut* 1975;16:683-8.
- 20 Kumar PJ, O'Donoghue DP, Stenson K, Dawson AM. Reintroduction of gluten in adults and children with treated coeliac disease. *Gut* 1979;20:743-9.
- 21 Marsh MN. Functional and structural aspects of the epithelial lymphocyte with implications for coeliac disease and tropical sprue. *Scand J Gastroenterol* 1985;20:55-75.
- 22 Leigh RJ, Marsh MN, Crowe P, Kelly C, Garner V, Gordon D. Studies of intestinal lymphoid tissue. IX. Dose dependent, gluten-induced lymphoid infiltration of coeliac jejunal epithelium. *Scand J Gastroenterol* 1985;20:715-9.

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