Cellular infiltrate of the jejunum after re-introduction of dietary gluten in children with treated coeliac disease

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SYNOPSIS Jejunal lamina propria plasma cells and eosinophils and intraepithelial lymphocytes were raised in coeliac children on gluten-containing diets, but only intraepithelial lymphocytes were increased in patients on gluten-free diets. In contrast, lamina propria lymphocytes were reduced in children with coeliac disease on gluten-containing diets but were normal in patients on gluten-free diets. In children with coeliac disease who were studied serially, lamina propria plasma cells and eosinophils and intraepithelial lymphocytes increased, and lamina propria lymphocytes decreased, within three months of the reintroduction of gluten to the diet. These observations are essentially similar to those made in the adult form of the disease and suggest that more than one type of immunological reaction is involved in the pathogenesis of the jejunal lesion.

Immunohistochemical (Douglas *et al*, 1970; Pettin-gale, 1971; Salvi-lahti, 1972; Shiner and Ballard, 1972; Doe *et al*, 1974) and ultrastructural (Shiner, 1973) studies suggest that humoral immune reactions play a part in the pathogenesis of the jejunal lesion of coeliac disease. Other observations, however, suggest that cell-mediated mechanisms involving either thymus-dependent (Ferguson, 1974) or non-immune lymphocytes (Ezeoke *et al*, 1974) are important. This local immune activity is reflected in an excess of intraepithelial lymphocytes (Ferguson and Murray, 1971) and abnormal numbers of lamina propria cells in the jejunum (Holmes *et al*, 1974; Montgomery and Shearer, 1974). Alterations in plasma and lymphocyte numbers might implicate humoral or cell-mediated mechanisms respectively, while an increase in granulocytes could support existing evidence that immune complexes are involved. The aim of this study, therefore, was to gain further information about these cell classes in the hope of clarifying the pathogenesis of the small bowel lesion of childhood coeliac disease and to see if this differed from that found in adult patients.

Patients and methods

In the group of patients studied a past diagnosis of

coeliac disease had been based on suggestive clinical and biochemical evidence but without jejunal biopsy confirmation. The children had been managed on a gluten-free diet and the majority were continuing on this regime at the time of study. Because of this unsatisfactory basis for the original diagnosis and in order to advise on a rational future treatment it was necessary to confirm or exclude the diagnosis of coeliac disease by jejunal biopsy. This in most instances required appraisal of jejunal morphology after reintroduction of gluten to the diet.

PATIENTS

Twenty-two children who had previously been diagnosed tenuously as having coeliac disease without histological confirmation between the ages of 0·5 and 5 years, mean 2 years, were included in the study at which time the mean age was 11·5 years, range 5-16·5 years. A thorough assessment of the diet was made in all patients by the same dietitian. Sixteen had been on a gluten-free diet from 1·5 to 13 years, mean 6·9 years, and six admitted to consuming varying amounts of gluten immediately before this study for three months to 12 years, mean 5·3 years. Jejunal biopsies were obtained at the beginning of the investigation. If the initial biopsy showed villous atrophy the diagnosis of coeliac disease was accepted. If, in contrast, the first biopsy

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was normal, the patient was instructed to take a diet containing at least 10 g of gluten as natural wheat protein per day for a period of three months, after which the patient was investigated again. Thus in 16 children biopsies were obtained while on gluten-free and, after three months, on gluten-containing diets.

METHODS

Jejunal biopsies were obtained by Crosby Capsule in fasting subjects from the duodenojejunal flexure under fluoroscopic control. Tissue was processed routinely and sections 5 microns thick were cut and stained with haematoxylin and eosin. Lamina propria cells were counted at a magnification of 1000 over an area of 0.25 mm² from representative zones between the muscularis mucosae and epithelium, results being expressed as cells per square metre (Savilahti, 1972). Particular note was made of the numbers of plasma cells, lymphocytes, eosinophils, and neutrophils. Intraepithelial lymphocytes were also counted, results being expressed as lymphocytes per 100 epithelial cells (Ferguson and Murray, 1971). Villous heights were measured using a micrometer eye piece (Stewart *et al*, 1967).

The Student *t* test was used for statistical analysis.

Results

JEJUNAL MORPHOLOGY

Of the six subjects who were on a gluten-containing

diet, two had villous atrophy (villous heights 0 and 120 microns) and have coeliac disease, while the remaining four had a normal villous structure (villous height, mean $\pm$ standard error (SE) 413 $\pm$ 33.1 microns), excluding the diagnosis of coeliac disease. Of the 16 on an alleged gluten-free diet, 13 had a normal initial biopsy (villous heights, mean $\pm$ SE 387 $\pm$ 15.7 microns). After three months on a gluten-containing diet 10 of these had a significant mucosal lesion (villous heights, mean $\pm$ SE 119 $\pm$ 28.5). Three, however, remained normal (villous heights, mean $\pm$ SE 444 $\pm$ 23.5) and have continued to show no evidence of coeliac disease after 24 months on a substantial daily intake of gluten. The other three patients with abnormal jejunal morphology (villous heights, mean $\pm$ SE 212 $\pm$ 65.7 microns) while on alleged gluten-free diets which on further investigation were found to contain small amounts of gluten showed increased morphological deterioration after three months on a 10 g gluten per day diet (villous heights, mean $\pm$ SE 120 $\pm$ 62 microns).

Therefore, of the 22 subjects studied, 15 have proven coeliac disease based on the jejunal mucosal response to gluten. The remaining seven had a normal mucosa and showed no evidence of coeliac disease even after a substantial intake of gluten ranging from 24 months to 12 years. The cause of these subjects' past gastrointestinal symptoms is not known but they clearly do not have coeliac disease.

Subjects	No.	Lamina propria ¹			Intraepithelial lymphocytes ²
		Plasma cells	Lymphocytes	Eosinophils	
Coeliac					
No gluten	13	3019 $\pm$ 363	1562 $\pm$ 158	379 $\pm$ 75	39 $\pm$ 3.5
Gluten	13	5536 $\pm$ 359	947 $\pm$ 92	797 $\pm$ 105	59 $\pm$ 1.5
Controls					
No gluten	3	2185 $\pm$ 128	1476 $\pm$ 115	337 $\pm$ 53	30 $\pm$ 1.0
Gluten	3	2376 $\pm$ 565	1423 $\pm$ 130	435 $\pm$ 105	31 $\pm$ 0.6
Controls (Total)	10	2531 $\pm$ 203	1382 $\pm$ 55	320 $\pm$ 54	30 $\pm$ 2.6

Table I Jejunal infiltrate in coeliac children and controls before and after reintroduction of dietary gluten

Means $\pm$ SE

¹Lamina propria cells expressed as cells per mm²

²Intraepithelial lymphocytes expressed as lymphocytes per 100 epithelial cells.

	No.	Villous heights	Lamina propria ¹			Intraepithelial lymphocytes ²
			Plasma cells	Lymphocytes	Eosinophils	
Coeliac villous atrophy	18	128 $\pm$ 23	5286 $\pm$ 298	985 $\pm$ 88	750 $\pm$ 83	59 $\pm$ 2.3
Coeliac normal villous morphology	10	379 $\pm$ 18	2829 $\pm$ 166	1636 $\pm$ 98	263 $\pm$ 47	35 $\pm$ 2.9
Controls	10	423 $\pm$ 17	2531 $\pm$ 203	1382 $\pm$ 55	320 $\pm$ 54	30 $\pm$ 2.6

Table II Relationship of jejunal infiltrate to villous structure

^{1, 2} See footnotes to table I.

Fig 1

Fig 2

In view of their completely normal jejunal morphology, subsequent good health, and, in particular, the absence of gastrointestinal disturbance for many years before this study, it was considered reasonable to accept them as controls.

LAMINA PROPRIA CELLS (see tables I and II and figs 1-3)

Plasma cells

Numbers of these cells in coeliac patients on a gluten-free diet did not differ significantly from

Figs 1-3 Cellular infiltrate of the lamina propria (LP) in children with coeliac disease on gluten-free (GFD) and gluten-containing (GCD) diets. Cells expressed as numbers per square millimetre.

controls. In contrast, they were raised in patients on gluten-containing diets compared to controls ($P < 0.002$). In the coeliacs studied serially, there was a significant increase in plasma cells after three months on a gluten-containing diet ($P < 0.002$). Coeliac biopsies with normal villous morphology and controls had the same numbers of plasma cells but both contained fewer than coeliac biopsies with villous atrophy ($P < 0.002$; $P < 0.002$).

Lymphocytes

Numbers of lymphocytes in coeliac patients on a gluten-free diet did not differ significantly from controls. In contrast, these cells in coeliac patients on gluten-containing diets were decreased compared to controls ($P < 0.01$). In the coeliac patients studied serially there was a significant fall in numbers after three months on a gluten-containing diet ($P < 0.01$). Coeliac biopsies with normal villous morphology and controls had the same numbers of lymphocytes but both contained more than coeliac biopsies with villous atrophy ($P < 0.002$; $P < 0.05$).

Eosinophils

Numbers of eosinophils in coeliac patients on a gluten-free diet did not differ from controls. In contrast, they were increased in coeliac patients on gluten-containing diets compared with controls

Fig 4 Intraepithelial lymphocytes expressed as lymphocytes per 100 epithelial cells: ● normal villous morphology; ▲ villous atrophy. Horizontal lines represent 2 standard deviations above and below the mean.

($P < 0.01$). In coeliac patients studied serially there was an increase after three months on a gluten-containing diet ($P < 0.01$). Coeliac biopsies with normal villous structure and controls had the same number of eosinophils but both had fewer than coeliac biopsies with villous atrophy ($P < 0.01$; $P < 0.01$).

Neutrophils

Small numbers of neutrophils were seen in seven of the 15 biopsies from coeliac patients on gluten-containing diets. They were not found in biopsies from patients on gluten-free diets or controls.

INTRAEPITHELIAL LYMPHOCYTES (see tables I and II and fig 4)

These cells were increased in coeliac patients on both gluten-containing and gluten-free diets compared with controls ($P < 0.002$; $P < 0.05$). In patients studied serially there was a significant increase after three months on a gluten-containing diet ($P < 0.002$). Coeliac biopsies with normal villous morphology and controls had the same numbers of intraepithelial lymphocytes but both had fewer than coeliac biopsies with villous atrophy ($P < 0.002$; $P < 0.002$).

In the three serially studied subjects who had normal villous morphology on both gluten-free (villous heights, mean $\pm$ SE 415 ± 32 microns) and gluten-containing diets (villous heights, mean $\pm$ SE 444 ± 23.5 microns) there was no change in lamina propria or intraepithelial cell numbers.

Discussion

A possible explanation for the reduction in lamina

propria lymphocytes after the reintroduction of dietary gluten in this study as noted in untreated adult patients (Holmes *et al*, 1974) is that under the antigenic stimulus of gluten, sensitized lymphocytes transform into plasma cells (Roitt, 1971). Such transformation would lead to the associated increase in plasma cells found in our children and previously studied adults (Holmes *et al*, 1974; Montgomery and Shearer, 1974; Lancaster-Smith *et al*, 1975).

An alternative reason for the depletion of lamina propria lymphocytes would be increased migration of these cells into the epithelial cell layer, thus giving rise to the excess of intraepithelial lymphocytes found in the currently studied children and in adult coeliac disease (Ferguson and Murray, 1971; Holmes *et al*, 1974; Montgomery and Shearer, 1974; Lancaster-Smith *et al*, 1975). The increased loss of lymphocytes from the epithelium into the gut lumen in untreated coeliac patients (Weetman *et al*, 1974) would support this hypothesis.

The increase in eosinophils confirms by quantitative methods the impression that these cells increase in coeliac children challenged with gluten (Shiner and Ballard, 1972). In adults, similar observations have been made (Pettingale, 1971; Lancaster-Smith *et al*, 1975) although other workers have not found a significant jejunal eosinophilia (Montgomery and Shearer, 1974; Holmes *et al*, 1974). The small numbers of neutrophils in some coeliac children in this study after gluten ingestion agrees with other results in children (Shiner and Ballard, 1972) and adults (Doe *et al*, 1974; Lancaster-Smith *et al*, 1975). Both eosinophil and neutrophil granulocytes are attracted by immune complexes (Kay, 1970). Our results might thus indirectly support the opinion that immune-complexes are formed in the jejunum at some stage in the pathogenesis of the mucosal lesion (Shiner and Ballard, 1972; Doe *et al*, 1974).

The effect of gluten on the jejunal inflammatory infiltrate in childhood coeliac disease is therefore essentially the same as in adult patients (Holmes *et al*, 1974; Montgomery and Shearer, 1974; Lancaster-Smith *et al*, 1975). In children a prolonged gluten-free diet results in a virtually normal infiltrate of the jejunum in association with a return to normal villous structure. This contrasts with adult patients on long-term treatment in whom abnormalities of the infiltrate remain in the majority of cases despite only minor abnormalities of villous morphology (Holmes *et al*, 1974; Lancaster-Smith *et al*, 1975). One explanation for this would be that adult patients adhere less strictly than children to a gluten-free diet. Alternatively, it could be that in adults with prolonged exposure to gluten before treatment the mucosa is more severely damaged and thus less capable of returning completely to normal. Neverthe-

less it is clear that in both childhood and adult forms of coeliac disease many cell types are involved in the inflammatory response to gluten, and this is compatible with current knowledge and opinion (*Lancet*, 1974) that more than one immunological mechanism may be responsible for the jejunal lesion of coeliac disease.

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