

# Cellular infiltrate of jejunal biopsies in adult coeliac disease in relation to gluten withdrawal<sup>1</sup>

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**SUMMARY** A comparison has been made of inflammatory cell counts in the lamina propria and epithelium of jejunal biopsies in 11 patients with adult coeliac disease with those found in 12 control subjects. In the coeliac patients, there were significant increases in the numbers of total cells, plasma cells, and intraepithelial lymphocytes, but a significant reduction in lamina propria lymphocytes. Following clinical improvement on a strict gluten-free diet, significant changes in cell counts occurred, but with the exception of lymphocytes in the lamina propria, the counts were still abnormal. Analysis of five patients in whom the biopsy improved to near normal morphology and of six in whom there was no such improvement showed that significant falls in plasma cells and rises in lymphocytes in the lamina propria could occur without improvement in other morphological appearances. These results seem relevant to the problem of diagnosing coeliac disease in patients who, on gluten withdrawal, show an unequivocal clinical response, but no gross morphological improvement in the jejunal biopsy. On the basis of the observed changes in cell counts, there seems little justification in questioning the diagnosis of coeliac disease in such patients.

A feature of the jejunal biopsy found in adult coeliac disease is the presence of a chronic inflammatory cell infiltrate (Paulley, 1954; Rubin, Brandborg, Phelps, and Taylor, 1960; Anderson, 1960; Thurlbeck, Benson, and Dudley, 1960). In the lamina propria, this consists mainly of plasma cells while in the epithelium it is almost exclusively made up of lymphocytes. Following treatment with a gluten-free diet these cells decrease in numbers (Yardley, Bayless, Norton, and Hendrix, 1962; MacDonald, Brandborg, Flick, Trier, and Rubin, 1964; Ferguson and Murray, 1971; Fry, Seah, McMinn, and Hoffbrand, 1972) while, on reintroduction of gluten, either by direct instillation into the small bowel (Rubin, Brandborg, Flick, Phelps, Parmentier, and Van Neil, 1962; Shmerling and Shiner, 1970) or by oral feeding (Bayless, Rubin, Topping, Yardley, and Hendrix, 1970) they again appear in increased numbers. From such observations and from the known involvement of plasma cells and lymphocytes in humoral and cellular immunity, it has been postulated that gluten may provoke an immunological reaction in the small intestine of patients with

coeliac disease and that this reaction may play a part in the genesis of the mucosal lesion (Bayless *et al*, 1970). In addition, Austad, Cornes, Gough, McCarthy, and Read (1967) have suggested that hyperactivity of lymphoid cells in the mucosa could be relevant to the increased incidence of malignancy reported in the disorder (Harris, Cooke, Thompson, and Waterhouse, 1967).

Recently, in two studies of coeliac disease, intraepithelial lymphocytes were counted in jejunal biopsies (Ferguson and Murray, 1971; Fry *et al*, 1972) but there has been no similar study of the various cells found in the lamina propria. This paper reports the results of quantitation of cells in the epithelial cell layer and lamina propria of biopsies from control subjects and of patients with adult coeliac disease. The latter were studied both before and at intervals after gluten withdrawal and in these patients cell counts were correlated with morphological responses to a gluten-free diet.

## Materials and Methods

### SUBJECTS

Jejunal biopsies from 11 well documented patients with adult coeliac disease were obtained both before

<sup>1</sup>Presented in part at the British Society of Gastroenterology meeting, Cardiff, 1973.

Received for publication 23 January 1974.

and after gluten withdrawal, and in four of the patients a further biopsy was performed whilst on the diet. Strict adherence to the diet was assessed by personal interview with each patient on several occasions. All showed an unequivocal clinical response, but the series was deliberately selected so that it contained two groups of individuals, one group of five patients in whom gross jejunal morphology was improved to near normal subsequent to gluten withdrawal (group A) and one group of six patients who showed no such improvement (group B). This made available for study relatively normal and abnormal specimens of jejunal mucosa. The first jejunal biopsies in the gluten-free period were obtained at an average time of two years and nine months after gluten withdrawal (range eight months to seven years and nine months). No further change occurred in gross jejunal morphology of the four patients who had a second biopsy while on a gluten-free diet at times varying from nine months to seven years and four months after their first biopsy under treatment.

The control group consisted of 12 individuals who had normal biopsies and showed no evidence of organic bowel disease after full investigation.

#### MORPHOLOGY

Sections 5  $\mu$  thick, stained with haematoxylin and eosin, were examined. The following cells were counted in the lamina propria: total cells made up of plasma cells, lymphocytes, reticulum cells, eosinophils, and 'other cells' (fibroblasts, endothelial cells, and unclassifiable cells), all of which were separately counted. In the epithelial cell layer, lymphocytes were quantitated. These various cells were counted under high power with the aid of a graticule placed in the eyepiece of the microscope.

Cells in an area of 0.05 mm<sup>2</sup> of lamina propria were studied at a distance of not more than 150  $\mu$  from the mucosal surface, while in addition, a length of epithelium of 1660  $\mu$  was scanned. Cell counts in the lamina propria were expressed as cells per mm<sup>2</sup> and in the epithelium as cells per mm length of epithelium. Only counts from the first biopsies performed during the gluten-free period were used for statistical comparison. The results were analysed by the Student *t* test.

#### Results

There were no significant differences in mean counts of reticulum cells, eosinophils, or 'other cells' between controls and patients with adult coeliac disease either before or after gluten withdrawal or between treated and untreated patients and these cells are not further considered. The means, 2 SD range, and results of statistical analysis for total cells, plasma cells, and lymphocytes in the lamina propria and intraepithelial lymphocytes in controls and patients are shown in table I. Table II shows the separate results for patients in groups A and B, whilst figures 1 and 2 show the counts for lymphocytes and plasma cells in the lamina propria and intraepithelial lymphocytes in all of the coeliac biopsies studied in relation to the time of gluten withdrawal. Mean cell counts for patients in groups A and B were not statistically different before the withdrawal of dietary gluten.

#### LAMINA PROPRIA

##### Total cells

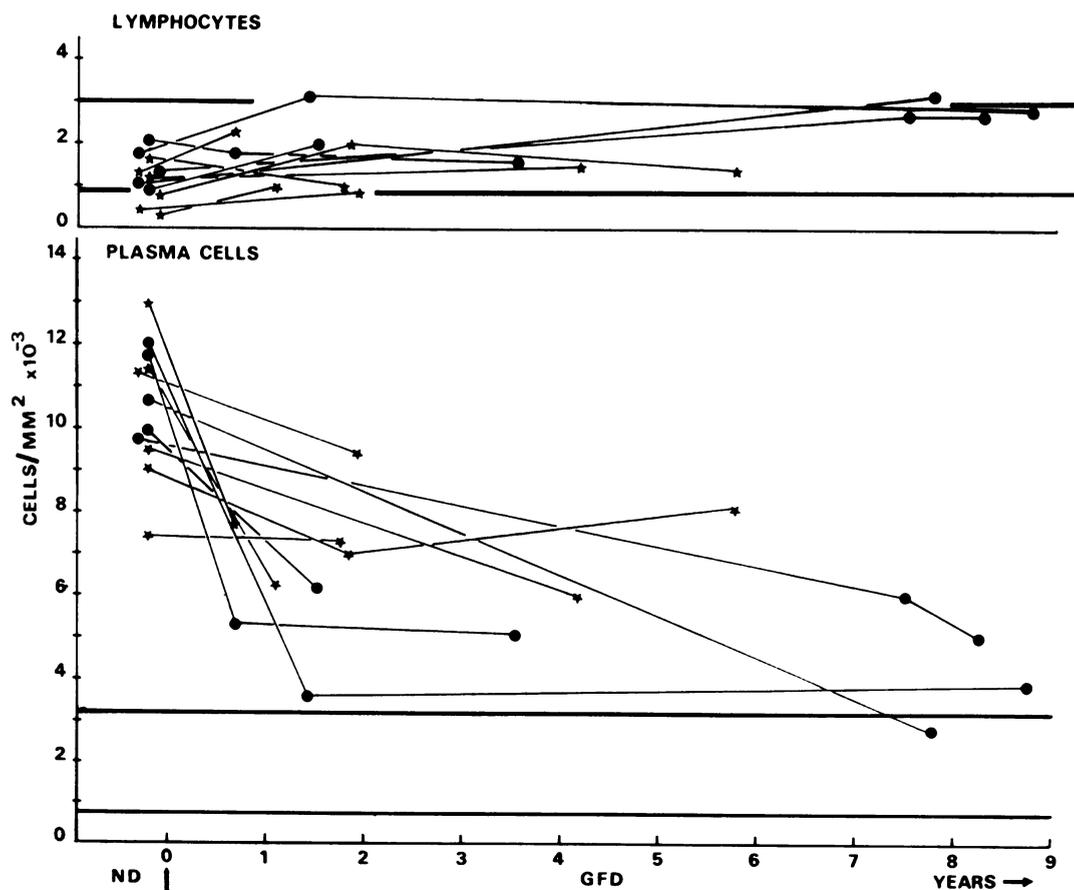
There was a significant increase in the mean total cell counts within the lamina propria for coeliac

		Total Cells	Plasma Cells	Lymphocytes	
				Lamina Propria	Epithelium
Controls	Mean	5702	1974	1948	42
	SD	1068	620	526	12
Adult coeliac disease Normal diet	Mean	13412	10498	1172	134
	SD	2134	1592	514	40
Gluten-free diet	Mean	9584	6148	2004	94
	SD	1072	1840	846	39
Controls	P	<0.001	<0.001	<0.01	<0.001
Adult coeliac disease (normal diet)					
Controls	P	<0.001	<0.001	NS	<0.001
Adult coeliac disease (gluten-free diet)					
Adult coeliac disease (normal diet)	P	<0.001	<0.001	<0.01	<0.05
Adult coeliac disease (gluten-free diet)					

Table I Mean counts, standard deviations, and P values for total cells, plasma cells, and lymphocytes in the lamina propria and lymphocytes in the epithelium

		Total Cells	Plasma Cells	Lymphocytes	
				Lamina Propria	Epithelium
<i>Adult coeliac disease (normal diet)</i>					
Group A	Mean	14268	10764	1416	152
	SD	2372	1040	276	37
Group B	Mean	12700	10276	970	120
	SD	1206	2016	448	39
Group A	v	NS	NS	NS	NS
Group B	v	NS	NS	NS	NS
<i>Adult coeliac disease (gluten-free diet)</i>					
Group A	Mean	8992	4788	2540	103
	SD	678	1514	682	45
Group B	Mean	10120	7280	1464	87
	SD	1268	1248	596	35
Group A	v	NS	<0.05	<0.05	NS
Group B	v	NS	<0.05	<0.05	NS
Group A (normal diet)	v	<0.05	<0.01	<0.05	NS
Group B (gluten-free diet)	v	<0.05	<0.05	NS	NS
Group B (normal diet)	v	<0.05	<0.05	NS	NS
Group B (gluten-free diet)	v	<0.05	<0.05	NS	NS

**Table II** Mean values, standard deviations, and p values for cell counts in patients with adult coeliac disease with improved jejunal biopsy to near normal on gluten-free diet (group A) and in patients without such improvement (group B)



**Fig 1** Lymphocytes and plasma cells in the lamina propria expressed as cells/mm<sup>2</sup> × 10<sup>-3</sup> of lamina propria for two groups of patients with adult coeliac disease (group A, ●) with improved jejunal morphology to near normal on gluten withdrawal and group B (\*) without such improvement. The initial counts while on a normal diet are shown and subsequently at different times indicated in years while on a gluten-free diet. The 2 SD range for the control group is indicated by the horizontal lines.

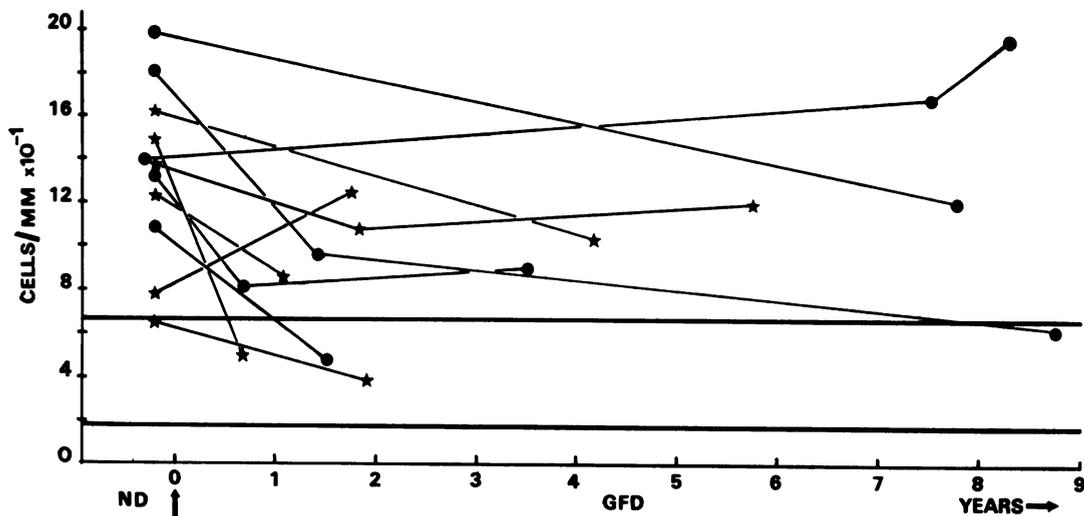


Fig 2 Intraepithelial lymphocyte counts, expressed as cells/mm<sup>2</sup> × 10<sup>-1</sup> length of epithelium. Legend otherwise as for figure 1.

patients taking a normal diet compared with controls ( $P < 0.001$ ). Although patients on a gluten-free diet have lower mean counts than those on a normal diet ( $P < 0.001$ ), they still have higher mean values than the controls ( $P < 0.001$ ). The mean cell counts of patients in both groups A and B were lower on a gluten-free than when on a normal diet ( $P < 0.05$ ) and whereas group A patients showed a greater mean fall on treatment, a comparison of the two groups while on a gluten-free diet showed no significant difference in their counts.

#### Plasma cells

The changes in plasma cell counts were similar with the exception that on a gluten-free diet the mean number of plasma cells in those patients (group A) showing histological improvement were significantly lower than those patients (group B) showing no such response ( $P < 0.05$ ).

#### Lymphocytes

Mean lymphocyte counts were reduced in patients on a normal diet compared with the controls ( $P < 0.01$ ). After gluten withdrawal, the mean counts were higher ( $P < 0.01$ ) and then did not differ significantly from the control individuals. On a gluten-free diet the mean cell counts in group A patients were higher than those on a normal diet ( $P < 0.05$ ) and were also greater than the mean counts in group B patients ( $P < 0.05$ ). In group B patients the mean cell counts increased on gluten withdrawal but the difference was not significant.

#### Epithelial lymphocytes

There was a higher mean count of lymphocytes in biopsies from patients on a normal diet compared with controls ( $P < 0.001$ ) and though the mean counts were lower ( $P < 0.05$ ) on a gluten-free diet they were still greater than the controls ( $P < 0.001$ ). Neither group A nor group B patients showed significantly lower counts in the gluten-free diet biopsies than in the normal diet biopsies. On a gluten-free diet, although group A patients had lower mean counts than those in group B this difference was not significant.

#### SERIAL STUDIES

Figures 1 and 2 show that in all but one patient there was an excess of plasma cells in the lamina propria up to nine years on a gluten-free diet, and usually the lymphocytes in the epithelial cell layer remained abnormal. In contrast lamina propria lymphocytes became normal and remained so throughout the period of study.

#### Discussion

The origin of the lymphocytes and plasma cells in the small intestine is not fully understood. They may arise locally *in situ* or invade from a source elsewhere. Thus experimentally following antigenic stimulation it has been found that immunoblasts formed in tissues remote from the gut will home to the lamina propria of the small bowel to produce plasma cells (Hall and Smith, 1970; Hall, Parry, and Smith,

1972). In coeliac disease the occurrence of serum antibodies to gluten (Taylor, Thomson, Truelove, and Wright, 1961; Heiner, Lahey, Wilson, Gerrard, Schwachman, and Khaw, 1962; Ferguson and Carswell, 1972) suggests that this antigen could gain access to the lymphoid tissue throughout the body. As a result of such antigenic stimulation, immunoblasts might appear in the efferent lymph of lymph nodes and a proportion of these home to the lamina propria of the small intestine and give rise to increased plasma cells. Treatment with a gluten-free diet by removing the antigenic stimulus would reduce this cell traffic and so reduce the numbers of plasma cells.

To explain the various changes in cell numbers found in the lamina propria and epithelium local factors need to be considered. For example, the local antigenic stimulus of gluten might bring about the transformation of lymphocytes into plasma cells. Such a transformation can occur (Roitt, 1971) and this would provide some explanation for the decreased number of lymphocytes and the raised levels of plasma cells found in the lamina propria. On removal of gluten from the diet the process would be reversed, lymphocytes would reaccumulate and plasma cells decrease, as we have found. Another explanation for the decreased number of lymphocytes in the lamina propria is loss into the gut lumen via the epithelial cell layer. Lymphocytes within this layer could be extruded along with the enterocytes which are lost in increased numbers in coeliac disease (Croft, Loehry, and Creamer, 1968), although evidence for the extrusion of lymphocytes is debatable (Pink, Croft, and Creamer, 1970). Also the hypothesis would require lymphocytes to pass from the lamina propria to the epithelium, the evidence for which is still not strong (Meader and Landers, 1967; Toner and Ferguson, 1971).

The persistence of abnormal numbers of cells in the mucosa for so many years after gluten withdrawal is puzzling. One obvious explanation would be that patients are continuing to ingest gluten sufficient to stimulate the immune system but not sufficient to produce clinical deterioration or indeed gross morphological abnormalities in the jejunal mucosa. This possibility can never be entirely ruled out. Alternatively, the persistence of the mononuclear cells could be explained if the mucosa were so severely damaged by long exposure to gluten that even after the institution of a gluten-free diet its normal microstructure was not completely restored, leading to abnormalities of cell traffic just on mechanical grounds. Furthermore, if there is recognition of cells between tissues as postulated by Hall *et al* (1972), damage to these could explain the

prolonged abnormal densities of cells in mucosa which appears virtually normal otherwise.

Finally, there was no correlation between clinical response to a gluten-free diet and restoration of villi in the proximal jejunum, which has been the experience of others (Doniach and Shiner, 1960; Buchan and Gerrard, 1962; Yardley *et al*, 1962; Shiner, 1963; Ruffin, Kurtz, Borland, Bain, and Roufail, 1964; Bolt, Parrish, French, and Pollard, 1964). Therefore, it is of some importance that on gluten withdrawal those patients who showed no appreciable change in gross morphology, ie, group B patients, showed significant changes in numbers of total cells and plasma cells in the lamina propria. Thus on this criteria they were gluten sensitive, suggesting that these two groups of patients are more homogenous than is often claimed.

The Endowment Fund for Medical Research of the United Birmingham Hospitals supported G.K.T.H. and P.L.S. G.K.T.H. was also initially in receipt of a grant from the Medical Research Council.

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