

T cell depletion in untreated adult coeliac disease

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SUMMARY The proportional and absolute numbers of circulating thymus dependant lymphocytes (T cells) were reduced in untreated patients with coeliac disease but were normal after treatment with a gluten free diet. There was an inverse correlation between circulating T cell numbers and jejunal intraepithelial lymphocytes. This evidence suggests a possible role for T cells in the pathogenesis of coeliac disease and is a further example of disturbed cell mediated immunity in this condition.

Thymus dependant lymphocytes (T cells) may be involved in the pathogenesis of the jejunal lesion in coeliac disease (Ferguson, 1974). In addition, impaired function of circulating lymphocytes, probably T cells, occurs in this disorder (Blecher *et al.*, 1969; McClaurin *et al.*, 1971). The numbers of circulating T lymphocytes in coeliac disease might have an important bearing upon these phenomena. The aim of this study, therefore, was to quantitate peripheral blood cells that spontaneously form rosettes with sheep red cells which are considered to represent the T cell subpopulation (Jondal *et al.*, 1972; Wybran *et al.*, 1973). The findings have been correlated with dietary treatment and lymphocyte populations of the jejunal mucosa in an attempt to elucidate the possible role of T cells in coeliac disease.

Methods

LYMPHOCYTE SEPARATION

Defibrinated blood was diluted by adding 3 parts phosphate buffered saline (PBS) to 1 part blood, layered on to a sodium metrizoate-Ficoll solution (Nyegaard & Co. As., Oslo) and centrifuged at $650 \times g$ for 30 minutes at 20°C. The separated lymphocytes were now washed twice in Eagle's medium at 4°C and finally resuspended in Eagle's medium to an approximate concentration of 2×10^7 per ml.

ROSETTE TEST

Sheep red blood cells (SRBC) were washed twice in PBS at 4°C and finally resuspended in Eagle's

medium to a concentration of approximately 40×10^6 cells/ml. The remainder of the test was performed according to the method of Wybran (Wybran *et al.*, 1973). The numbers of rosette forming lymphocytes were expressed both as a percentage of the total lymphocyte population and as absolute numbers per ml of blood, calculated from the formula, white cells per ml of blood \times percentage lymphocytes \times percentage rosette forming cells. A rosette was defined as a lymphocyte surrounded by at least 3 SRBC. The yield of lymphocytes was consistently greater than 80%. Groups of patients and controls were always studied concurrently.

JEJUNAL BIOPSIES

These were obtained from 19 patients by Crosby capsule from the duodenojejunal flexure coincident with blood lymphocyte studies. Sections 5μ thick were stained with haemotoxylin and eosin. Jejunal lamina propria lymphocytes were counted and expressed as lymphocytes per mm^2 of mucosa (Lancaster-Smith *et al.*, 1975). Intraepithelial lymphocytes were counted and expressed as lymphocytes per 100 epithelial cells (Ferguson and Murray, 1971). At least 500 epithelial cells were counted.

PATIENTS

The groups studied were 24 patients with treated adult coeliac disease, 29 untreated patients, and 39 controls of comparable age and sex distribution and nutritional status. Nine patients were studied both before and after treatment with a gluten free diet. Ten patients with malabsorption due to conditions other than coeliac disease were also studied (Table 1). The degree of malabsorption in this group was, in general, more severe than in the patients with untreated coeliac disease.

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Cause of malabsorption	Patients (no)
Small bowel bypass for obesity	3
Chronic pancreatitis	2
Jejunal diverticula	1
Tropical sprue	1
Partial gastrectomy	1
Biliary obstruction	1
α -chain disease	1
	10

Table 1 Cases of malabsorption not due to coeliac disease

	Nos	T cells (%)	T cells/ml*	Total lymphocytes/ml*
Coeliacs				
Untreated	29	42.4 ± 1.7	577 ± 51	1340 ± 95.5
Treated	24	62 ± 1.6	1050 ± 95.5	1674 ± 141
Controls	39	59 ± 1.5	942 ± 58	1604 ± 86

Table 2 Peripheral blood T cells in adult coeliac disease

Means ± SE; * Multiply by 10³.

Results

The proportional and absolute numbers of T lymphocytes in the peripheral blood of patients with untreated ACD were decreased when compared with treated patients ($P < 0.002$, $P < 0.002$) controls ($P < 0.002$, $P < 0.002$) and patients with malabsorption not due to coeliac disease ($P < 0.002$, $P < 0.05$). Treated patients had the same proportional and absolute numbers of T cells as controls (Figs 1 and 2). Unlike T lymphocytes, total peripheral

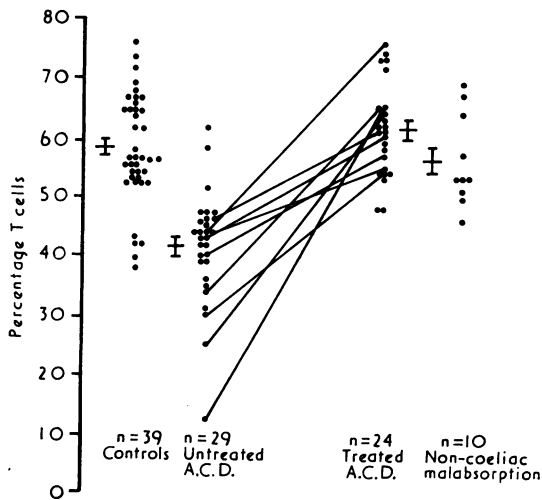


Fig. 1 Rosette-forming T lymphocytes in peripheral blood as a percentage of total lymphocytes.

blood lymphocytes did not differ significantly in the four groups (Table 2). All nine patients studied both before and after treatment with a gluten free diet showed a proportional rise in T cells ($P < 0.001$) and seven of nine an increase in absolute cell numbers ($P < 0.025$) (Figs 1 and 2).

Jejunal intraepithelial lymphocyte counts showed an inverse correlation with the proportional ($P < 0.001$) (Fig. 3) and absolute ($P < 0.01$) peripheral blood T cell counts. In contrast, there was no correlation between peripheral blood T cells and jejunal lamina propria lymphocytes or between total

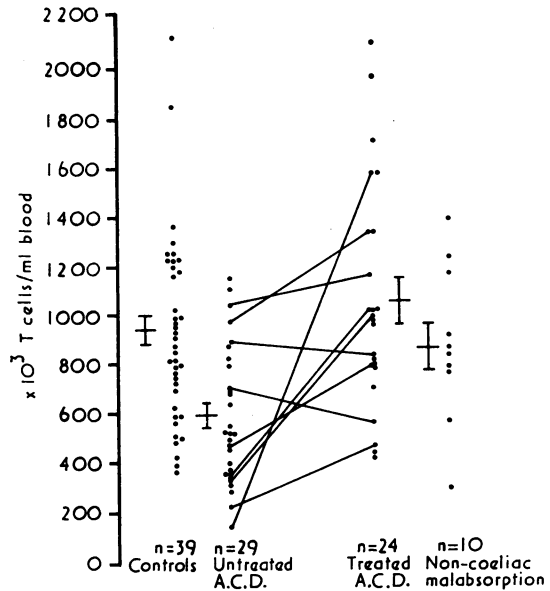


Fig. 2 Rosette-forming T lymphocytes in peripheral blood as absolute numbers.

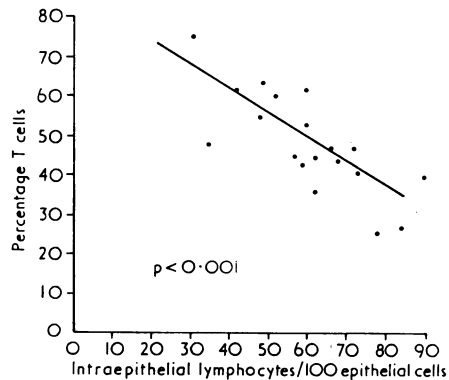


Fig. 3 Correlation between peripheral blood T lymphocytes and jejunal intraepithelial lymphocytes.

peripheral blood lymphocytes and jejunal intra-epithelial or lamina propria lymphocytes.

Discussion

The depletion of circulating T cells in untreated coeliac disease might be due to impaired production, increased sequestration in lymphoreticular tissue (the most likely areas being small bowel mucosa and mesenteric lymph nodes), or excessive loss into the gut lumen. Which one or combination of these is responsible is not clear but the return to normal levels after treatment with a gluten free diet implies that it is a secondary phenomenon. In untreated coeliac disease there is an excess of lymphocytes in the epithelial cell layer of the jejunum (Ferguson and Murray, 1971; Ferguson *et al.*, 1974) and increased loss of lymphocytes into the gut lumen (Weetman *et al.*, 1974), both of which are also reversed by treatment. If the depletion of circulating T cells be due to these mechanisms, it would imply a selective sequestration or loss of T cells compared with non-T cells, but whether this is so remains unknown. However, in immunohistochemical studies of the jejunal mucosa in coeliac disease, cells of lymphocyte proportions staining with anti-immunoglobulin sera, which are presumed to be B lymphocytes, have been found only in the lamina propria and not in the epithelial cell layer (Rubin *et al.*, 1965; Lancaster-Smith *et al.*, 1974). This indirectly suggests that the great majority of intraepithelial lymphocytes are non-immunoglobulin bearing T cells. The inverse correlation between the degree of circulating T cell depletion and the increase of intraepithelial lymphocytes would support this. Alternatively, both phenomena might be two unrelated features of untreated coeliac disease (Marsh, 1975).

Circulating lymphocytes from patients with untreated coeliac disease show reduced blast transformation in response to the gliadin fraction of gluten when compared with treated patients (Asquith, 1974). This, Asquith believes, is due to large numbers of gliadin sensitized lymphocytes leaving the blood and sequestering in the small bowel mucosa of untreated patients. Our findings would support this hypothesis and suggest that the majority of lymphocytes attracted to the gut are T cells. The lymphoreticular dysfunction (McCarthy *et al.*, 1966), impaired blast transformation produced by non-specific mitogens (Blecher *et al.*, 1969; Asquith, 1974), reduced cytotoxic capacity of lymphocytes against tumour cells *in vitro* (McClaurin *et al.*, 1971), and the increased incidence of gut lymphomas found in coeliac disease (Austad *et al.*, 1967; Harris *et al.*, 1967) are considered to be evidence of cell mediated immunodysfunction in this

condition. The depletion of T cells, which are of prime importance in cell mediated reactions, could therefore have some bearing on these deficiencies and it might be that the return to normal numbers of T cells after a gluten free diet is the reason for the reduced incidence of gut neoplasms in treated coeliacs compared with untreated patients (Harris *et al.*, 1967; Holmes *et al.*, 1974).

In conclusion, it seems possible that T lymphocytes may be involved in the pathogenesis of coeliac disease and that their depletion in untreated patients may partly explain the associated cell mediated immunodysfunction. However, a role for T cells does not necessarily imply that cell mediated mechanisms *per se* are involved in the small bowel lesion of coeliac disease because T cells are also important in humoral immune mechanisms, some of which have been implicated in the pathogenesis of this disorder (Lancet, 1974).

The finding of normal numbers of circulating T lymphocytes in patients with malabsorption from conditions other than coeliac disease, whose general state was comparable with the patients with untreated coeliac disease, makes it extremely unlikely that the T cell depletion in this latter group is a manifestation of malabsorption *per se*.

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