Case report

Coeliac disease with severe hypogammaglobulinaemia

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SUMMARY A patient with severe late onset primary hypogammaglobulinaemia developed coeliac disease. The case illustrates that coeliac disease can occur in the virtual absence of local antibody production by plasma cells in the mucosa of the small bowel. Furthermore, our inability to demonstrate specific cellular immunity to a subfraction of gluten raises doubts about the relevance of immunological reactions in the pathogenesis of coeliac disease.

There are two main hypotheses to explain the aetiology of coeliac disease. Bronstein *et al.*¹ suggested that the disease was caused by enzymatic failure to degrade gluten with a consequent production of peptides which were toxic to the small intestinal epithelial cells. This hypothesis does not exclude a secondary role for immunological reactions in the pathogenesis of the condition. The currently more favoured view² is that the intestinal lesion is initiated and perpetuated by an immunological reaction. This is supported by the finding of numerous plasma cells in the intestinal lesions of coeliac disease.³ Cellular immune mechanisms may also play a part in causing damage to the mucosa, and it has been suggested that the lymphocytes of coeliac patients may be sensitised to certain subfractions of gluten.^{4,5} The strong association with the HLA-DW3 histocompatibility antigen is further evidence that the disease has an immunological basis.

The presence of complement and its breakdown products in the mucosal lesions of coeliac patients suggests that the damage is mediated by an Arthus reaction.⁶ The specificity of the antibody involved is not known but is presumed to be directed against a component of gluten. However, the case described here shows that antibody mediated reactions are not essential in the pathogenesis of coeliac disease.

Case report

LT developed pneumonia when aged 43 years, having previously shown no undue susceptibility to

infections. After this he suffered with recurrent episodes of bronchitis and sinusitis. Hypogammaglobulinaemia was diagnosed at 53 years and he was treated with regular weekly injections of gamma-globulin. Thereafter, he did well and only had one or two attacks of bronchitis requiring antibiotics during the subsequent 15 years.

For the past 25 years he has suffered with mild recurrent eczematous dermatitis on the fingers and seborrhoeic dermatitis in the groins. At the age of 55 years he developed a small patch of vitiligo over the sternum. During the first few months of 1978 he experienced a number of anaphylactic reactions to his gammaglobulin injections, although he has had no reactions for the past two years.

In 1974 (aged 63 years) he began passing three to four steatorrhoeic motions a day, although he was not losing weight. Physical examination at this time showed no abnormality and the following investigations confirmed malabsorption: the five day faecal fat collection on a 100 g fat diet per day was 9.3 g/day. The red cell folate was 76 ng/ml (normal 145-450 ng/ml) and the peripheral blood film showed a mild macrocytosis. The serum calcium was 2.2 mmol/l (normal 2.19-2.66 mmol/l). A standard xylose absorption test (25 g oral dose) showed a five hour urinary excretion of 15 mmol (normal > 33 mmol). The serum vitamin B₁₂ was normal at 340 pg/ml and the Schilling test was normal. A pentagastrin stimulation test showed normal gastric acid and intrinsic factor production. Barium meal showed a malabsorption pattern in the small bowel and a fine nodular pattern in the terminal ileum characteristic of nodular lymphoid hyperplasia. A

Lundh test showed normal pancreatic function with an intestinal juice trypsin level of 33 IU. A jejunal aspirate contained no giardia parasites and when cultured aerobically and anaerobically for bacteria grew $<10^4$ bacteria/ml (normal $<10^4$).

The immunological tests showed a severe hypogammaglobulinaemia and normal cellular immunity

Table Immunological tests on patient LT

	<i>IU/ml</i>	<i>95% range of results for normal adults (IU/ml)</i>
Immunoglobulins		
Serum	IgG	41
	IgA	<0.07
	IgM	<0.12
	IgE	7
Jejunal juice	IgG	0.25
	IgA	<0.03
	IgM	0.06
Isohaemagglutinin titre		
Anti A	1:64	≥ 1:32
Anti B	1:32	≥ 1:16
<i>E. coli</i> antibody titre		
	1:4	≥ 1:64
Blood lymphocytes		
T cells (%)	828–1701/μl	1300–3500/μl
B cells (%)	71	50–74
	8	4–12
Delayed hypersensitivity skin tests		
Candida	} Positive	
Mumps		
PPD		
Lymphocyte transformation		
to PHA	3528	4000–10 000 cpm
HLA typing A1, W30/B8, 18/CW5/DRW3/DW3		

(Table), and he was classified as having adult onset primary hypogammaglobulinaemia. His lymphocytes carried the HLA—A1, B8, DW3 haplotype which is commonly seen in coeliac patients.

JEJUNAL HISTOLOGY AND CLINICAL PROGRESS

The initial jejunal biopsy (Fig. 1a) taken in October 1974 shows subtotal villous atrophy with crypt hyperplasia. Inflammatory cells were increased in the lamina propria but only occasional plasma cells were noted. The intraepithelial lymphocyte count was increased (57 lymphocytes/100 epithelial cells; $N = <30$). Surface enterocytes showed focal flattening and degeneration. The patient was started on a strict gluten free diet in November 1974 and within three weeks he was passing normal motions once a day (Fig. 2). He was still asymptomatic when readmitted in May 1975 for a repeat jejunal biopsy (Fig. 1b). This showed considerable improvement with mild partial villous atrophy. The surface cells were columnar and the intraepithelial lymphocyte count was 33 lymphocytes/100 epithelial cells. Plasma cells were not indentified in the lamina propria. He remained on a gluten free diet and was asymptomatic when readmitted in March 1977 for a gluten challenge to confirm the diagnosis. The pre-challenge jejunal biopsy showed partial villous atrophy and no significant change was seen in a biopsy taken 21 hours after gluten. A post-gluten biopsy at 45 hours showed a lymphoid follicle and was inadequate for detailed analysis.

When the gluten challenge failed to confirm the diagnosis of gluten enteropathy, he was allowed to

Fig. 1 (a) Initial jejunal biopsy showing subtotal villous atrophy with crypt hyperplasia. H and E, $\times 95$. (b) Jejunal mucosa showing improvement in appearance of villi after six months on a gluten-free diet. H and E, $\times 95$. (c) Jejunal mucosa showing relapse to subtotal villous atrophy after three months on an unrestricted diet. H and E $\times 95$. (Original magnifications.)

Fig. 2 Relationship between diet, symptoms, and mucosal changes.

return to a free diet in November 1977. However, within four weeks he developed steatorrhoea with at least four bowel motions a day. He was readmitted for a jejunal biopsy in February 1978, having lost about half a stone in weight. The jejunal biopsy (Fig. 1c) was similar to that taken in October 1974 with severe villous atrophy and an increased intra-epithelial lymphocyte count (58 lymphocytes/100 epithelial cells). No plasma cells were seen and immunoperoxidase staining for intracellular IgG, IgA, and IgM was negative. Having established a diagnosis of gluten enteropathy, he was again put on a gluten free diet and within a few weeks was passing one normal motion a day. He has since been mainly asymptomatic with occasional bouts of diarrhoea when he relaxes his diet.

SPECIAL STUDIES

Gluten challenge

A gluten challenge test was performed in March 1977, the patient having been on a strict gluten free diet for 16 months. Twenty grams of cooked gluten was taken orally after an initial jejunal biopsy. Additional jejunal biopsies were taken at 21 hours and 45 hours after gluten. Serum was obtained on each occasion to measure total haemolytic complement, IgE and C3, but these showed no significant changes. Immunofluorescence studies on the three jejunal biopsies taken during this study showed no cellular staining for IgA and IgG and only one or two cells in each biopsy stained for IgM. The connective tissue of the lamina propria showed bright staining for IgG, which probably originated from the gamma-globulin injections. Connective tissue staining with

antisera to C3 was faint in the pre-challenge mucosa and increased in both of the post-challenge biopsies. Staining for secretory piece was normal. Electron-microscopy of the pre- and post-challenge biopsies was similar and showed normal enterocytes. No plasma cells were seen, but lymphocytes, macrophages, and eosinophils were plentiful.

Mucosal immunoglobulin production in vitro

We measured immunoglobulin production *in vitro* by the patient's jejunal mucosa in order to check that small amounts of immunoglobulin were not being produced by cells that did not have the usual morphological appearances of plasma cells. Using the technique of Oldham *et al.*,⁷ the jejunal biopsy taken in February 1978 during relapse was cut into three pieces (weight 1, 3.0, and 2.8 mg) and cultured separately *in vitro* to assess local immunoglobulin production. No detectable IgA or IgM—that is, <2ng/mg tissue—was produced by the samples at any time over six days. In contrast, normal rectal biopsies continued to produce 30–>1000 ng/IgA per mg of tissue/day on the sixth day of culture (see Oldham *et al.*⁷). Some IgG was present in the culture supernatant at 24 hours but rapidly decreased, and was unrecordable on the fifth day. This indicated that the IgG originated from outside the gut and was not synthesised by cells in the mucosa.

Specific immunity to gluten

Lymphocyte transformation studies to a gluten subfraction were performed in July 1978, when the patient was asymptomatic on a gluten free diet. Peripheral blood lymphocytes obtained from a Ficoll/trisil density gradient were cultured at 1×10^6 /ml in Dulbecco's modified medium and 10% human AB serum with 10, 50, and 100 μ g/ml final concentration of the Oxford B2 fraction of gluten. This fraction, which has been previously shown to stimulate lymphocyte transformation in patients with coeliac disease, is a subfraction of Frazer's fraction III.⁵ 3 H thymidine (1 μ Ci) was added at 40 hours and the cultures harvested using a Scatron automatic harvester eight hours later. Other cultures were left for five days before 3 H thymidine was added and the cultures harvested 16 hours later. The results showed that there was no increased uptake of 3 H thymidine in the cultures containing B2 fraction compared with control cultures at either 48 hours or 5½ days. However, these data should be interpreted with caution, as no positive controls were used.

Skin tests

An intradermal skin test with 0.1 ml of the Oxford B2 fraction, together with an appropriate control, was performed as recommended by Anand *et al.*¹⁰ At

six hours, the B2 fraction produced an area of erythema of 15 mm diameter which then gradually faded and disappeared by 24 hours. The control test site showed a 3 mm reaction at six hours and then faded rapidly. The skin tests were repeated one month later when the B2 fraction gave a response of 10 mm in diameter, but this time biopsies of the test areas for both the B2 fraction and the control were obtained at six hours. The histology showed an inflammatory reaction, with mononuclear cells and neutrophils in both the control and B2 fraction test site. There was also no significant difference in the number of basophils present in both the control and the test site and immunofluorescent studies showed no accumulation of C3.

Discussion

Coeliac disease has been previously suspected in a few patients with primary hypogammaglobulinaemia, but has never been satisfactorily confirmed by showing a relapse on a normal diet.⁸⁻⁹ The necessary criteria for the diagnosis of gluten enteropathy have been fulfilled in this patient by demonstrating both histological and clinical improvement after gluten withdrawal, followed by relapse on a normal diet, and then improvement again after gluten withdrawal. Apart from an occasional IgM-containing cell, immunofluorescent and immunoperoxidase staining together with electronmicroscopy failed to demonstrate the presence of immunoglobulin containing plasma cells in jejunal biopsies from this patient. It is difficult to interpret the negative findings after the gluten challenge and we might have seen changes if we had waited longer than 48 hours before repeating the jejunal biopsy.

Although Anand *et al.*¹⁰ found a good correlation between coeliac disease and positive skin tests to the B2 fraction of gluten, the interpretation of the skin test in this patient is not straightforward, as positive skin tests are also associated with other inflammatory bowel disorders.¹¹ Although the timing of the skin reaction in our patient was consistent with an Arthus reaction, this was not confirmed by the histology and immunofluorescent findings.

It has been suggested that the increase in epithelial lymphocytes means that the mucosal damage could be mediated by a cellular immune reaction.¹² Intra-epithelial lymphocyte counts were raised in our patient during relapse. However, Guix *et al.*¹³ suggest that this has little significance in the pathogenesis, as, in absolute terms, the number of intra-epithelial lymphocytes does not alter during relapse and remission. Moreover, we found no evidence that

peripheral blood lymphocytes from the patient were sensitised to the Oxford B2 fraction. Although the significance of lymphocyte transformation tests in this context remains contentious, Bullen and Losowsky,¹⁴ using a macrophage inhibition factor (MIF) test, have demonstrated the presence of lymphocytes sensitised to Frazer's fraction III in the blood of patients with coeliac disease. However, late skin reactions at 48 and 72 hours were not seen when the B2 fraction was used for skin testing.

This case demonstrates that the pathogenesis of coeliac disease does not necessarily depend upon the presence of a plasma cell infiltrate into the mucosa. It is also unlikely in this case that an Arthus type reaction is involved in the mucosal lesions. However, we cannot completely exclude a role for antibody as some IgM and IgG was detectable in the jejunal juice and the presence of isohaemagglutinins in the serum indicated some residual capacity to make IgM antibodies. Nevertheless, the IgM in the jejunal juice probably came from the bile and the IgG from the gammaglobulin injections. This patient, like many others with adult onset primary hypogammaglobulinaemia, had a few immunoglobulin-containing cells within the gut mucosa,¹⁵ although we were able to demonstrate only one or two IgM-containing cells in some of the biopsies. The apparent accumulation of C3 in the mucosa after gluten challenge is unexplained but could be due to activation of the alternate complement pathway by gluten.¹⁶ However, this may only have reflected increased transudation of C3 from serum, as the immunofluorescent studies were done on fixed tissues.

We have found no evidence that immunological mechanisms are involved in this patient's coeliac disease. This suggests that the link between the HLA-DW3 histocompatibility marker and coeliac disease may not involve immunological reactions.¹⁷ Although immunological reactions may play a part in the pathogenesis of coeliac disease in most patients, this case shows that other non-immunological factors are likely to be of primary importance.

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