Ultrastructural analysis of plasma cells in coeliac patients

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SUMMARY By accurate morphometry the length of the rough endoplasmic reticulum (RER) was estimated in electron micrographs of a population of jejunal plasma cells in biopsies obtained from normal volunteers, untreated coeliac disease patients, and coeliac disease patients treated for one year on a gluten free diet. Increase in length or hypertrophy of the endoplasmic reticulum is an indication of increased protein synthesis of the cell (Ghadially, 1977). An increase compared with normal length was demonstrated in the mean length of the plasma cell rough endoplasmic reticulum in plasma cells obtained from both groups of coeliac patients. This estimate of increased protein production—in this case immunoglobulin—indicates an increase in the immunological activity of plasma cells even after treatment and suggests not only a reaction to dietary gluten but probably to other antigens also.

There has been ample evidence over the past 20 years that an immunological mechanism is involved in some way in coeliac disease (Rubin *et al.*, 1965; LePore, 1958; Kivel *et al.*, 1964; Harris *et al.*, 1967; Asquith *et al.*, 1969; Blecher *et al.*, 1969; Brown *et al.*, 1969; Hobbs *et al.*, 1969; Douglas *et al.*, 1970; Wall *et al.*, 1970; Rubin, 1971; Strober *et al.*, 1974; Holmes *et al.*, 1976; Lancaster-Smith *et al.*, 1976). It is known that there is an increase in the number of plasma cells in the jejunal mucosa of coeliac patients and, after treatment on a gluten free diet, the proportion of these cells returns to normal or almost normal (Asquith, 1974; Holmes *et al.*, 1974; Lancaster-Smith *et al.*, 1976). There have also been qualitative and quantitative studies related to the proportions of different types of immunoglobulin-producing-plasma cells in the lamina propria (Douglas *et al.*, 1970; Søltoft, 1970; Pettingale, 1971; Lancaster-Smith *et al.*, 1974; Savilahti, 1974), but hitherto there have been no attempts to quantify the ultrastructural features of plasma cells in an objective manner so that an estimation of their functional state can be made in coeliac patients both before and after treatment.

Method

The plasma cells studied were in jejunal biopsies obtained in the usual way under radiological control

from normal volunteers and patients with coeliac disease whose diagnosis was based on clinical and morphological grounds—that is, the presence of malabsorption, total villous atrophy, and crypt hyperplasia of the jejunal mucosa when the patient was first seen, clinical and morphological improvement after one year on a gluten free diet, and the re-appearance of morphological abnormalities after further gluten challenge. The biopsies of the patients on a gluten free diet were subjectively indistinguishable from normal using the usually accepted criteria (Whitehead, 1973).

A portion of each of the biopsy specimens, taken at random from the whole specimen after removal of about half the specimen for enzyme analysis, was fixed in 5% glutaraldehyde, processed and embedded in Araldite (Ciba-Geigy) resin. Sections were cut at approximately 0.5 microns using an LKB ultra microtome and floated onto water. The thinner sections were identified by their silver-grey colour and were produced entirely by chance. Some were picked up randomly as they floated on the water bath and placed on copper grids. They were examined using a Philips 201C electron microscope at a magnification of $\times 1939$ (determined by reference to particles of known size). Only those fields between grid bars could be examined and this was determined during preparation without observer choice. They were then photographed. In order to assess the activity of plasma cells the length of the endoplasmic reticulum (RER) was measured on the microphoto-

graphs. A grid was prepared which had five rows of 10 parallel lines of equal length placed in such a way that the end points of the lines of each row formed an equilateral triangle with the end points of the line immediately above. This grid was placed at random by dropping it over the microphotographs (Fig. 1) and the number of intercepts of the lines with the RER were counted. Thus the cells and the position of the counting grid over the plasma cells were selected randomly. The final length of the RER was obtained by multiplying the number of intercepts (I) by a tenth of the overall width of the grid in millimetres (W) and by a further 10^3 to convert this into microns, this then being divided by the final magnification (M) of the photographic enlargement—

that is, $\frac{I \times W \times 10^3}{M}$. Only one plane of each

plasma cell was measured, providing that it contained a recognisable nucleus. It was assumed that

the RER, though a series of long curved lines, was in fact made up of a series of short straight lines randomly distributed (Weibel, 1969). The number of cells which needed to be measured to obtain accurate results was found by the 'quotient of accumulative addition of values' (Dunnill, 1968) in which the length of the RER was related to the maximum diameter of the cell. In Fig. 2 it can be seen that no less than 17 cells had to be measured in each case to get accurate results. The maximum diameter reference point was chosen purely for convenience of measurement but the cell area determined by point counting would be as reliable and probably show that fewer cells need be counted as it is a more stable denominator. In effect, therefore, the accuracy of this estimate of RER was probably increased as more intercepts were counted. A total of 246 cells was measured from different groups of jejunal biopsies: 100 plasma cells from five normal biopsy specimens, 75 from biopsy specimens of four

Fig. 1 Grid placed over an electronmicrophotograph of a plasma cell to count the number of intercepts with the RER. $\times 2000$ (original magnification).

Fig. 2 Graph of the quotient of accumulative addition of values between the number of intercepts with the RER and the diameter of the cell (L. RER/CD). The quotient remains stable after 17 plasma cells examined.

untreated coeliac patients, and 71 from biopsy specimens of four patients treated for one year on a gluten free diet. Statistical analysis was performed using the *t* test and carried out on a DEC 10 computer using programmes from the *Statistical Package for the Social Sciences* (Nie *et al.*, 1975).

Results

Results from different groups of biopsies were expressed as mean values and standard deviations (Table). Plasma cells from the group of normal subjects had a mean length of the RER of 41.64 microns, with a standard deviation (*s*) of 6.14. Those from untreated patients showed a mean of 61.74

(*s* = 10.93) and from treated patients a mean of 59.01 (*s* = 6.13).

Analysis between pairs of the groups showed that the length of RER in both the plasma cells from untreated coeliacs and from treated coeliacs was different from normal subjects at the 5% level conventionally chosen, the value of *t* for the first comparison in a two-tailed test being 3.51 (*P* < 0.01) and for the second 4.21 (*P* < 0.01 at 7 degrees of freedom). There was no significant difference between plasma cells from treated and untreated groups.

Discussion

In the lamina propria of jejunal mucosa of untreated coeliac patients there is a significant increase in the number of plasma cells and a decrease in the number of lymphocytes, but these return to normal or near normal after a gluten free diet (Asquith, 1974; Holmes *et al.*, 1974; Lancaster-Smith *et al.*, 1976).

Quantitative studies of the absolute numbers of immunoglobulin-containing cells in coeliac disease show a significant increase in IgA, IgM, and IgG producing cells compared with normal. The increase is greatest in IgA and IgM cells (Douglas *et al.*, 1970; Søltøft, 1970; Pettingale, 1971; Lancaster-Smith *et al.*, 1974; Savilahti, 1974).

These studies provide only part of the available information concerning the quantitative aspect of plasma cells in coeliac disease and objective data as to their immunological activity based on ultra-structural grounds are lacking. For instance, Shiner (1973) in a subjective study, suggested that dilatation of the RER in plasma cells in coeliac patients could be equated with an increase in their immunological activity, but this is contrary to the generally accepted view that dilatation of the RER in whatever cell

Table Mean values (*X*) and standard deviations (*s*) of the length of RER in normal subjects and coeliac patients

	<i>X</i>	<i>s</i>	Mean and SD from different group means
Normal subjects			
Case 1	38.85	21.18	
Case 2	32.47	16.70	
Case 3	47.74	22.30	<i>X</i> = 41.64
Case 4	46.11	16.44	<i>s</i> = 6.14
Case 5	43.03	16.48	
Untreated coeliac patients			
Case 6	55.01	17.02	
Case 7	54.35	18.38	<i>X</i> = 61.74
Case 8	59.89	19.70	<i>s</i> = 10.93
Case 9	77.71	18.25	
Treated coeliac patients			
Case 10	59.34	24.46	
Case 11	51.79	17.96	<i>X</i> = 59.01
Case 12	66.74	31.68	<i>s</i> = 6.13
Case 13	58.17	13.92	

t test

. untreated: *t* 3.51 d.f. 7 *P* < 0.01
. treated: *t* 4.21 d.f. 7 *P* < 0.01

type signifies some defect in the egress of secretory material (Ghadially, 1977). Few cells with dilated RER were seen in this study and were no more common in the coeliac group. It is known that the RER is the site of production of immunoglobulin in plasma cells and the length of the RER is an indication of activity of the cell (Ghadially, 1977). In this study the length of the RER was measured and used as an index of immunological activity. Though there is an overlap between the groups, based on the length of RER, there is an increase in the number of 'activated' cells when plasma cells from coeliac patients are compared with those of normal subjects.

This probably reflects the different distribution of the various stages in the continuum of plasma cell differentiation in this disease. It also demonstrates the involvement of a humoral immunological reaction as the protein produced (immunoglobulin) is presumably secreted. Cells from treated patients after one year on a gluten free diet also show evidence of activation, although the overall architecture of the jejunal mucosa is normal. This finding suggests that a humoral immunological reaction persists even in the absence of dietary gluten and is possibly an ongoing response to other antigens. A similar conclusion has been drawn by Baklien *et al.* (1977) based on a study of plasma cells both by their numbers in lamina propria determined by Ig class and their position in the mucosa. Prolonged antigenic stimulation and plasma cell activation are known to predispose to lymphoma in other organs where immunological mechanisms are involved in the disease process (Cox, 1964; Talal *et al.*, 1967). It is pertinent, therefore, that in the view of some (Holmes *et al.*, 1976; Thompson, 1976) a gluten free diet does not remove the liability to lymphoma in coeliac subjects.

According to these results we conclude that a humoral immunological reaction occurs in coeliac disease, and that a similar reaction is probably developed to antigens other than gluten, but this possibly non-specific reaction does not affect to any great extent the general morphology of the jejunal mucosa.

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