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Jejunal plasma cells and *in vitro* immunoglobulin production in adult coeliac disease

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SUMMARY

IgA, IgE, IgG and IgM plasma cells in small bowel mucosal biopsies from 15 controls, 16 untreated and 14 treated coeliac patients and five patients with selective serum IgA deficiency (four of whom also had coeliac disease) were quantified using an indirect immunoperoxidase technique. The IgA, IgG and IgM plasma cell counts were significantly increased in the untreated coeliac patients. The cell counts were intermediate in the treated coeliac group. These changes were in parallel to production *in vitro* of IgA and sIgA, IgG, and IgM by cultured mucosal biopsies from the same patients. The IgA deficient patients had very few mucosal IgA cells but elevated IgG and IgM plasma cell numbers; again these changes were reflected in the production *in vitro* of immunoglobulins. IgE plasma cell counts were very low in all patients and there were no differences between patient groups. The changes in cell counts and mucosal immunoglobulin production were not reflected in serum IgA, IgM and IgG concentrations but serum secretory IgA was significantly elevated in the untreated coeliac patients compared with controls, with the treated coeliac patients being intermediate. The raised mucosal plasma cell counts reflect the local mucosal production of immunoglobulin but not the immunoglobulin concentrations of serum, emphasising the importance of studying the immune function of the gut itself in coeliac disease rather than immunological abnormalities in serum.

Keywords plasma cells immunoglobulin production coeliac disease

INTRODUCTION

Many immunological abnormalities have been described in patients with coeliac disease. There are changes in serum concentrations of immunoglobulins (Immonen, 1967; Hobbs & Hepner, 1968; Asquith, Thompson & Cooke, 1969) including secretory immunoglobulin A (sIgA) (Asquith, Thompson & Cooke, 1971), changes in the immunoglobulin content of intestinal secretions (Lancaster-Smith *et al.*, 1974; Asquith, Thompson & Cooke, 1970; Douglas, Crabbe & Hobbs, 1970) and in the immune cell population of the small bowel mucosa (Ferguson, Asquith & Cooke, 1974; Holmes *et al.*, 1974). There are, however, problems in the estimation of intestinal immunoglobulins as proteolysis or contamination from other gastrointestinal secretions probably take place. It is widely assumed that the increase in plasma cell density in coeliac mucosa is responsible for the raised immunoglobulin concentrations reported in intestinal mucosa (Scott *et al.*, 1980) and serum (Pettingale, 1971) of coeliac patients and that serum food antibodies are produced by intestinal immune cells (Girard & De Kalbermatten, 1973).

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There is disagreement, however, regarding the absolute and relative change in each immunoglobulin class of plasma cell, with immunoglobulin A (IgA) plasma cell density, for example, being reported as higher (Scott *et al.*, 1980) or lower (Pettingale, 1971) than in controls.

Most studies of mucosal plasma cells have used immunofluorescent techniques. The immunoperoxidase method has advantages in that cell morphology can be clearly seen and intracellular immunoglobulin is easier to distinguish from extracellular immunoglobulin.

In order to clarify the situation and provide further information about some of these problems, we have studied the plasma cell population of jejunal mucosa by immunoperoxidase labelling in parallel with measurement of production *in vitro* of immunoglobulin by matched biopsies, and have measured the serum concentrations of immunoglobulins A, G and M and sIgA in the same patients.

MATERIALS AND METHODS

Patients studied

Control patients. Fifteen control patients were studied (mean age 35·6 years, range 18–75). Small bowel biopsies from each patient had a normal appearance on dissecting microscopy and histological examination. Eleven were subsequently found to have the irritable bowel syndrome. The remaining four had no evidence of gastrointestinal disease and had anaemia (two patients), multiple sclerosis (one) and fibrosing alveolitis (one). Serum samples were available from 11 of these patients. All the above were regarded as 'normal control patients'.

Serum samples were also obtained from eight patients with gastrointestinal disease ('disease controls'). Three patients had Crohn's disease of the distal small bowel, one recurrent intestinal giardiasis, one tuberculous peritonitis and three chronic pancreatitis. In each case, proximal small bowel histology was normal.

IgA deficient patients. Small bowel biopsies were obtained from five patients with selective deficiency of serum IgA. Four of these patients also had coeliac disease, of whom two were in relapse and two in remission at the time of biopsy. The fifth patient had normal small bowel histology.

Untreated coeliac patients. Sixteen patients had presumed coeliac disease (mean age 40·9 years, range 17–68). In each case, the patients were taking gluten-containing diets at the time of biopsy and all biopsies had a flat mosaic appearance on dissecting microscopy and sub-total villous atrophy on histological examination. All 16 have shown symptomatic improvement on a gluten-free diet and 14 have had repeat small bowel biopsies, all of which have shown histological improvement to normal or near-normal. Two patients have yet to be re-biopsied. Serum samples were available from nine of these patients.

Treated coeliac patients. Biopsies were obtained from 14 patients with coeliac disease in remission (mean age 39·7 years, range 17–68). All had shown a good clinical and morphological response to a gluten-free diet: in 12 patients the post-treatment small bowel biopsies were normal on histology and, in the remaining two, minor villous changes were present in places. The average duration of gluten-free diet was 4 years. Serum samples were obtained from seven of these patients and an additional 13 patients who had shown a histological and clinical response to a gluten-free diet.

Biopsies

Multiple peroral small bowel biopsies were taken, with informed consent, from the region of the duodeno-jejunal junction by a hydraulic multiple biopsy capsule (Quinton, Flick & Rubin, 1962). In all subjects, this was done as part of the routine diagnostic procedure. These studies were approved by the local Ethical Committee.

Mucosal immunoglobulin production

Production of IgA, sIgA, IgG and IgM by small bowel biopsies during *in vitro* culture was estimated as described previously (Wood *et al.*, 1986). Briefly, small bowel mucosal biopsies were maintained in an organ culture system (Howdle *et al.*, 1981) for 24 h. Ig contents of the culture media were

estimated by sensitive enzyme-linked immunosorbent assay (ELISA) methods for total IgA, IgG, and IgM, using human serum as the standard, and sIgA using a colostral IgA standard, as previously described (Wood, Trejdosiewicz & Losowsky, 1987). ELISA for total IgA, IgG and IgM were performed by coating flat-bottomed ELISA plates (Dynatech, Billinghurst) with goat polyvalent antiserum to human Ig (Sigma Chemicals, Poole) at 400 ng/well. After incubation with sample or standard, goat anti-human IgA, IgG or IgM alkaline phosphatase (AP) conjugate (Sigma) was added to each well and enzyme activity detected with *p*-nitrophenyl phosphate substrate (Sigma). Secretory IgA was measured on plates coated with 200 ng/well sheep anti-human secretory component (Seward, Bedford) as the solid-phase antibody, with goat anti-IgA AP-conjugate as the detecting antibody. Monomer IgA has been shown not to interfere in this assay even when present in large excess (Wood *et al.*, 1987). The immunoglobulin production was expressed as μg per mg of biopsy wet weight.

Immunoperoxidase labelling

Small bowel samples were taken from areas of jejunum adjacent to those used to study mucosal Ig production. Biopsies were fixed overnight in formalin, embedded in paraffin wax and 5 μm sections were cut perpendicular to the muscularis mucosae. The sections were mounted on glass slides and dried overnight at 37°C. After treatment with 1% hydrogen peroxide in methanol to block endogenous peroxidase activity, 0·1% trypsin solution was used to unmask antigen (Curran & Gregory, 1977). Labelling for IgA, IgE, IgG and IgM was carried out using the indirect immunoperoxidase technique (Burns, 1978) with Harris's haematoxylin counterstain.

Single batches of rabbit antisera to human colostral IgA, IgE, IgG and IgM (heavy chain-specific) were obtained from Dako Ltd (High Wycombe). Specificity was confirmed by immuno-electrophoresis and double diffusion against purified antigen and normal human serum, and by absorption experiments using the relevant antigen to abolish the reactivity on tissue sections (Heyderman, 1979). In view of the reported cross-reaction of some IgE antisera with IgA (Piris & Murdoch, 1981), the anti-IgE was pre-absorbed with excess colostral IgA. A range of dilutions of primary antisera was used for each tissue section (Scott *et al.*, 1984) to ensure consistency of cell counting. Swine anti-rabbit Ig peroxidase conjugate (Dako) was used as the second layer.

Cell counting

Plasma cells, identified morphologically, were counted by means of a Leitz projecting microscope. All plasma cells found between the muscularis mucosae and the epithelial surface of the bowel were counted for a fixed length of muscularis mucosae, amounting to four non-overlapping adjacent fields. The cell counts were expressed as number of plasma cells per mm length of muscularis mucosae. For each section, the average cell count obtained from three dilutions of primary antiserum was recorded.

Serum immunoglobulins

Serum samples were obtained from patients at the time of small bowel biopsy and stored at −20°C. Serum IgA, IgG and IgM were estimated by cellulose acetate electrophoresis and sIgA by ELISA, as described above (Wood *et al.*, 1987).

Statistics

Statistical significance was tested by the Mann Whitney *U*-test, and correlation using Spearman's rank correlation coefficient.

RESULTS

Plasma cell counts. The median cell counts for IgA, IgE, IgG and IgM plasma cells in the normal control patients were 73, 6, 23 and 40 per mm mucosal length (Table 1, Fig. 1), giving approximate ratios of IgA:IgE:IgG:IgM plasma cells as 12:1:4:7. In the untreated coeliac group, the median cell counts were 176, 6, 40 and 101 respectively (ratios of approximately 29:1:7:17). The plasma cell

Table 1. Jejunal plasma cell counts (median and range) in coeliac, normal control and IgA-deficient patients (cells/mm mucosal length)

	Control patients	Untreated coeliac patients	Treated coeliac patients	IgA-deficient patients
IgA	73 (53–148)	176 (113–321)	104 (58–143)	6 (1–40)
IgE	6 (1–13)	6 (0–32)	5 (1–18)	2 (1–9)
IgG	23 (13–38)	40 (22–52)	19 (11–65)	74 (40–125)
IgM	40 (24–70)	101 (75–193)	51 (14–98)	123 (54–151)

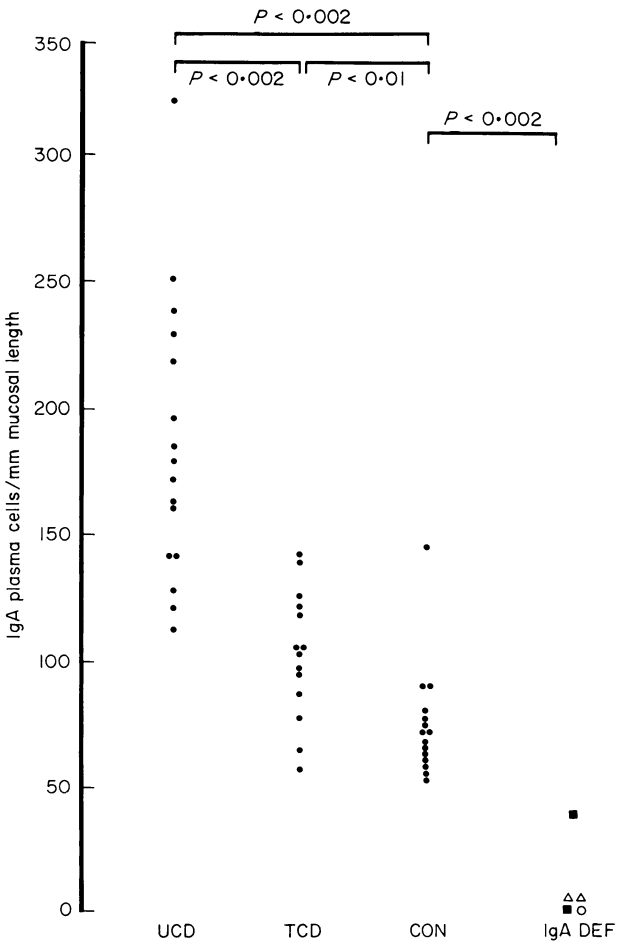

Fig. 1. Jejunal IgA plasma cell counts in untreated (UCD) and treated (TCD) coeliac, normal control (CON) and IgA-deficient (IgA DEF) patients (cells/mm mucosal length). IgA-deficient patients: (■) untreated coeliac disease; (△) treated coeliac disease; and (○) control patient.

counts were significantly higher than those of the normal controls ($P < 0{\cdot}002$) but, in the case of IgG cells, there was considerable overlap with the normal control group. In the treated coeliac patients, the median cell counts were 104, 5, 19 and 51 respectively (ratios of approximately 21 : 1 : 4 : 10; Table 1). The plasma cell counts were significantly lower compared with untreated coeliac patients ($P < 0{\cdot}02$) but, compared with normal controls, only the IgA plasma cell counts were significantly higher ($P < 0{\cdot}01$).

The IgA-deficient patients had very few IgA plasma cells present (median value 6/mm mucosal length). This was significantly less than the normal control group ($P < 0{\cdot}002$). By contrast, the IgG and IgM plasma cell counts of these patients were significantly elevated ($P < 0{\cdot}002$), the ratio of plasma cells being approximately 3 : 1 : 42 : 70 (Table 1).

Few IgE plasma cells were seen in the lamina propria of any of the patients and the plasma cell counts were similar in the control and patient groups (Table 1).

Immunoglobulin production in vitro. Small bowel biopsy samples from 12 of the 15 normal control patients were maintained *in vitro* for 24 h. The median amounts of total IgA, sIgA, IgG and IgM secreted by these biopsies were 1·24, 0·17, 0·46 and 0·19 μg/mg biopsy weight respectively (Table 2). The corresponding figures for biopsies from the 16 untreated coeliac patients were 3·3, 0·41, 1·2 and 0·98 respectively. The production of IgA, sIgA and IgM by the untreated coeliac biopsies was significantly greater than that of the control biopsies ($P < 0{\cdot}02$); that of IgG was not significantly increased ($0{\cdot}05 < P < 0{\cdot}1$). Biopsies from 11 of the 14 treated coeliac patients produced the following median amounts of Ig: 0·93, 0·19, 0·54 and 0·44 μg/mg biopsy weight. The amounts of IgA, sIgA ands IgG produced by the treated coeliac biopsies were not significantly different from those of the normal control biopsies but that of IgM was significantly greater ($P < 0{\cdot}02$). The treated coeliac biopsies produced less IgA, sIgA and IgM than the untreated coeliac biopsies ($P < 0{\cdot}02$); there was no difference in IgG production.

The biopsies from the IgA-deficient individuals produced significantly smaller amounts of IgA and sIgA than the control biopsies but significantly more IgM and IgG ($P < 0{\cdot}02$). These results for Ig production *in vitro* were similar to our previous findings (Wood *et al.*, 1986).

Serum Ig levels. There were no significant differences in the concentrations of serum IgA, IgG and IgM in the normal control and untreated coeliac patients. Serum IgA was marginally lower in the treated coeliac group than the untreated coeliac group ($P < 0{\cdot}05$). However, the concentrations of serum sIgA in nine of the untreated coeliac patients were significantly elevated compared with samples from 11 normal controls and 20 treated coeliac patients ($P < 0{\cdot}02$) (Fig. 2). Those of the treated coeliac patients were elevated when compared with the controls ($P < 0{\cdot}002$). In five coeliac patients, serum samples were obtained before and after treatment with a gluten-free diet for at least 6 months. In each case, the concentration of sIgA was lower after gluten elimination (Fig. 3).

Serum sIgA was undetectable in the five IgA-deficient patients. Three of eight bowel disease controls had elevated serum sIgA concentrations above the range found in normals (mean + 2 s.d.,

Table 2. *In vitro* immunoglobulin production (median and range) by jejunal biopsies from coeliac, normal control and IgA-deficient patients (μg/mg biopsy weight/24 h)

	Control patients	Untreated coeliac patients	Treated coeliac patients	IgA-deficient patients
IgA	1·24	3·3	0·93	0·02
	(0·3–2·9)	(0·42–25)	(0·25–6·7)	(0–0·9)
sIgA	0·17	0·41	0·19	0·002
	(0·09–0·63)	(0·12–1·64)	(0·06–0·29)	(0–0·4)
IgG	0·46	1·2	0·54	6·4
	(0·15–1·9)	(0·28–3·2)	(0·24–4·3)	(1·2–35)
IgM	0·19	0·98	0·44	1·3
	(0·08–0·51)	(0·23–5·3)	(0·13–1·6)	(0·68–2·4)

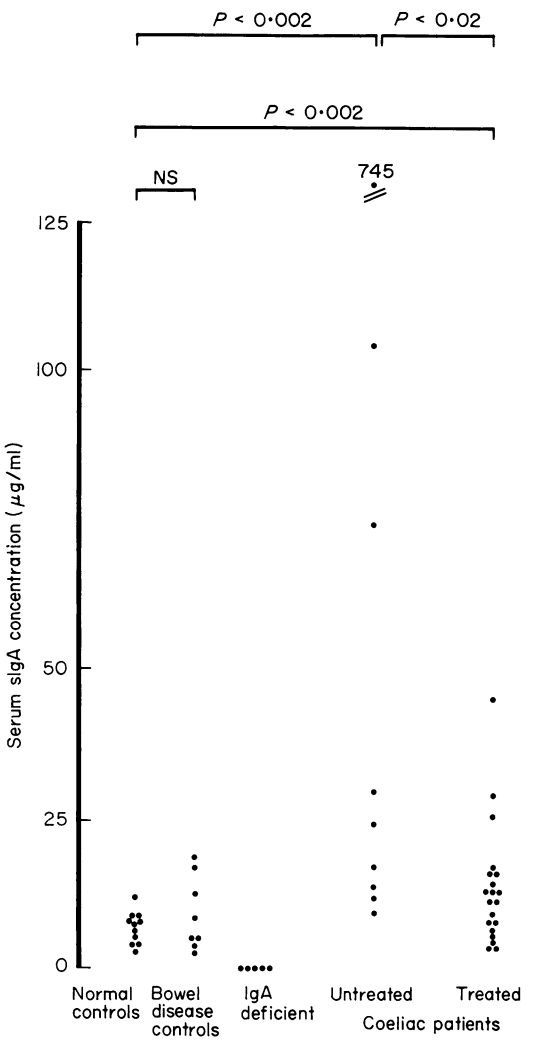

Fig. 2. Serum secretory IgA concentrations in coeliac and control patients (μg/ml).

12·0 μg/ml), but the values of the group did not differ significantly from those of the normal controls (Fig. 2). The patient (an untreated coeliac patient) with the highest serum sIgA also had an elevated serum IgA concentration.

Relationship between plasma cell counts, Ig secretion and serum Ig. Although there was no correlation between the plasma cell count and Ig production of paired biopsies within each group of patients, there was a significant positive correlation between the patient groups in the case of IgA (Fig. 4), sIgA and IgM, but not IgG (Table 3) with the untreated coeliac patients having the highest plasma cell counts and immunoglobulin production rates. The treated coeliac patients occupied an intermediate position and the normal control patients had the lowest plasma cell counts and immunoglobulin production rates.

For those patients in whom both biopsy and serum samples were available (11 normal controls, nine untreated and seven treated coeliac patients), there was no correlation between mucosal plasma cell counts and serum Ig concentrations of any class.

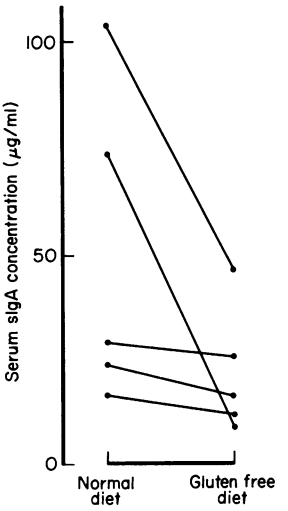

Fig. 3. Serum secretory IgA concentrations in five coeliac patients before and after a gluten-free diet.

Table 3. Correlation of *in vitro* immunoglobulin production (μg/mg biopsy weight) and plasma cell counts (cells/mm mucosal length) from paired jejunal biopsies

	Correlation co-efficient	*P* value
IgA	0·558	<0·001
sIgA	0·593	<0·001
IgG	0·122	NS
IgM	0·70	<0·001

DISCUSSION

We have demonstrated a large rise in IgA and IgM plasma cells in untreated coeliac mucosa, with a moderate increase in IgG cells. The changes reverted towards normal in treated coeliac mucosa. These findings confirm those of Scott *et al.* (1984) who used the peroxidase-anti-peroxidase (PAP) technique. They reported, however, higher absolute plasma cell counts, which may reflect a difference in the sensitivities of the two methods (Burns, 1975).

We did not find an increase in IgE plasma cells as reported by Scott *et al.* (1984) using PAP labelling, and Kumar, O'Donoghue & Lancaster-Smith (1979) who used immunofluorescence. However, other workers have also failed to demonstrate large numbers of mucosal IgE plasma cells, and have not reported significant increases in coeliac disease (Savilahti, 1972; Hobbs *et al.*, 1969). Other lamina propria cells (such as mast cells) contain IgE and are immunolabelled with anti-IgE antibodies, as has been pointed out by Brandtzaeg & Baklien (1976), who warned of problems in the interpretation of IgE immunolabelling of tissue sections. The mast cell population of the intestinal mucosa is expanded in coeliac disease (Strobel, Busuttil & Ferguson, 1983) and this may explain the apparent increase in IgE-positive cells reported by Kumar *et al.* (1979) and Scott *et al.* (1984). We have also previously found very low production *in vitro* of IgE by both normal and untreated coeliac mucosal biopsies (Wood *et al.*, 1986). These data suggest that IgE plasma cells do not play a major role in the pathogenesis of coeliac disease.

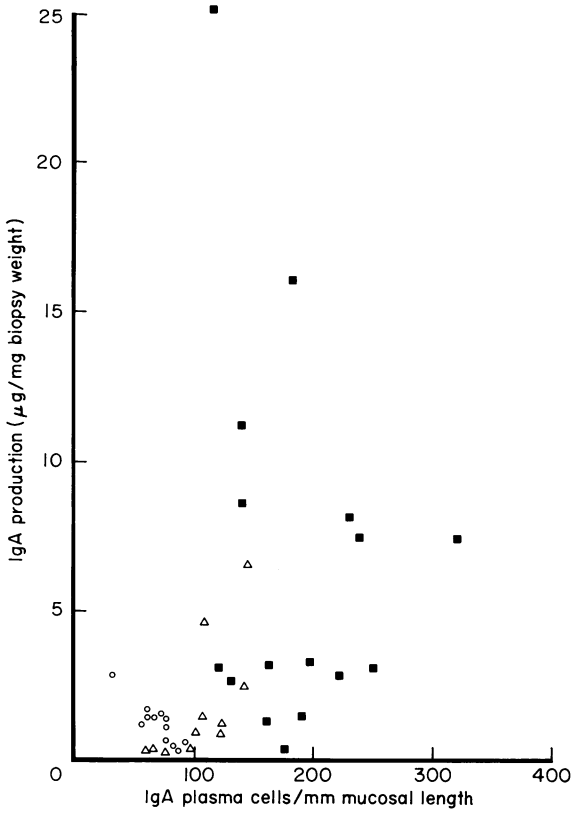

Fig. 4. Immunoglobulin production *in vitro* (μg/mg biopsy weight) and plasma cell counts (cells/mm mucosal length) from paired jejunal biopsies for IgA. (○) Normal controls; (△) treated coeliac; (■) untreated coeliac patients. $r_s = 0{\cdot}558$; $P < 0{\cdot}001$.

Our studies of Ig production *in vitro* demonstrate that the increase in lamina propria plasma cells in coeliac disease is accompanied by increased local mucosal production of Ig. These changes are most marked for IgA and IgM with only minor changes in IgG plasma cell counts, and no change in IgG production being observed. We have previously demonstrated that IgA and IgM release into culture medium from cultured jejunal biopsies remains constant for at least 72 h (Wood *et al.*, 1986), implying continued production by the intestinal mucosa (Kagnoff, Donaldson & Trier, 1972; Falchuk & Strober, 1974). However, the amount of IgG produced falls rapidly with time in culture (Wood *et al.*, 1986), and therefore probably represents passive diffusion of residual IgG from the tissue rather than *de novo* synthesis. The increase in local IgA and IgM synthesis and plasma cell counts probably accounts for the increase in extracellular collection of IgA and IgM described in coeliac mucosa (Scott, Scott & Losowsky, 1977; Doe, Henry & Booth, 1974). The lack of correlation between plasma cell counts and Ig production within each group of patients may be partly due to lack of precision in measuring very small amounts of immunoglobulin or to the small range within each group. Factors other than the number of mucosal plasma cells may also be involved in the local synthesis of Ig, such as the stimulated helper T cell population described by Malizia *et al.*, (1985).

In the IgA-deficient patients, the IgA plasma cells were extremely scanty with compensatory increases in IgM and particularly IgG plasma cells, with similar changes in the rate of local immunoglobulin production (Brandtzaeg, Fjellanger & Gjeruldsen, 1968; Brandtzaeg, 1971). The association of gluten sensitive villous atrophy with IgA deficiency has been described by a number of authors (reviewed by Asquith, 1974).

Of particular interest is the contribution of mucosal Ig production to the Ig profile of serum. It has been assumed that IgA and IgM from the intestinal mucosa enters the serum via the gut vessels or lymphatics. However, the increase in mucosal IgA and IgM plasma cells and local Ig production by the mucosa in this study was not accompanied by an increase in serum concentration of IgA (Immonen, 1967; Asquith *et al.*, 1969) or IgM and indeed the latter is believed to fall in untreated coeliac disease (Hobbs & Hepner, 1968; Asquith *et al.*, 1969). It thus seems unlikely that serum Ig concentrations accurately reflect the local mucosal production of Ig by the small intestine and most circulating Ig is probably derived from peripheral lymphoid tissue (Kutteh, Prince & Mestecky, 1982).

We have, however, found a substantial increase in serum sIgA in untreated coeliac patients which returns towards normal on gluten withdrawal. The intestine is a major site of sIgA production (Kutteh *et al.*, 1982) and we have demonstrated a significant increase in production of the secretory form *in vitro*. In one patient, a massive increase in serum sIgA was accompanied by elevated serum IgA as measured by a method which does not distinguish monomeric from polymeric IgA. This suggests that the elevated serum IgA seen in some coeliac patients may be due to a substantial rise in serum sIgA rather than monomeric IgA. The untreated coeliac patients had higher IgA plasma cell counts and local production of sIgA than treated coeliac and control patients, suggesting that the jejunal mucosa is in part responsible for the rise in serum sIgA.

Unfortunately, we have not been able to include data from non-coeliac patients with a flat jejunal mucosa for comparison in this study. Such patients are rare in adult practice in the UK, but we feel the comparison available between the coeliac patients and the control patients included here shows clearly the differences between the coeliac condition and normal.

Although we have shown that marked changes in mucosal plasma cell numbers and immunoglobulin production occur in coeliac disease, and it is tempting to postulate that such changes are primarily concerned in the pathogenesis of the disease, these could be purely part of a secondary immunological reaction occurring in the small intestinal mucosa as a result of the primary insult. This interpretation of our findings would be in accord with the case report of Webster *et al.* (1981) who described coeliac disease in a patient with severe hypogammaglobulinaemia.

We conclude that observations such as those reported here, and elsewhere (Wood *et al.*, 1986; Ciclitira *et al.*, 1986; Howdle, Bullen & Losowsky, 1982), emphasize the importance of studying the immune function of the intestinal mucosa in attempting to unravel the immunopathology of intestinal disease.

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