

Immunoglobulin secretion by isolated intestinal lymphocytes: spontaneous production and T-cell regulation in normal small intestine and in patients with coeliac disease

J E CRABTREE, R V HEATLEY, AND M L LOSOWSKY

From the Department of Medicine, St James's University Hospital, Leeds

SUMMARY The *in vitro* secretion of immunoglobulins by small intestinal lymphocytes isolated from 47 patients with normal histology and 23 patients with treated and untreated coeliac disease was examined using an enzyme linked immunosorbent assay. In control patients, duodenal lymphocytes spontaneously secreted higher levels of IgM than jejunal lymphocytes ($p < 0.05$). Significantly higher levels of both IgA ($p < 0.05$) and IgM ($p < 0.001$) were secreted by jejunal lymphocytes of 10 patients with untreated coeliac disease than cells isolated from normal jejunal tissue. IgM and IgA secretion by duodenal lymphocytes isolated from control patients was increased in a dose dependent manner by coculture with autologous peripheral blood T lymphocytes. This effect was not observed with jejunal lymphocytes of control or treated coeliac patients. Peripheral T-cells of untreated coeliac patients, however, showed significant helper effects ($p < 0.05$) for IgM and IgA secretion by autologous jejunal lymphocytes. The results suggest that jejunal lymphocytes of patients with untreated coeliac disease show major differences in their capacity to synthesise and secrete immunoglobulins *in vitro* and the enhanced secretion might result from changes in T-cell immunoregulatory function.

The mechanisms involved in the pathogenesis of coeliac disease are poorly understood. Although systemic cellular immune responses have been examined in detail,¹ investigations of mucosal immune responses have been limited. Immunohistological analysis of mucosal T lymphocytes has shown increased expression of stimulation markers and activation antigens^{2,3} and there have been varying reports of changes in plasma cell densities and isotype frequency.^{4,5} Alterations in mucosal architecture and surface to volume ratios associated with villous atrophy probably account for different results.⁷

Enhanced local secretion of immunoglobulins in coeliac patients has been demonstrated using *in vitro* organ culture systems^{6,9} and the deposition of immune complexes may be important in mediating mucosal damage.¹⁰ Contamination of mucosally synthesised immunoglobulins by serum derived immunoglobulins, however, can not be excluded in these studies. The isolation of lymphoid cells from

intestinal tissues by enzymatic digestion for short term *in vitro* culture^{11,12} allows examination of mucosal responses at the cellular level. There have, however, been only limited studies of the functional capacity of isolated human small intestinal lymphocytes^{13,14} and none pertaining to coeliac disease. In this study we have utilised cell isolation techniques, first to examine intestinal immunoglobulin secretion using isolated cell populations, and second to investigate the regulatory role of T lymphocytes on humoral intestinal responses. Abnormalities in humoral responses in coeliac disease may be secondary to abnormalities in T cell immunoregulation.

Methods

PATIENTS AND TISSUES

Control patients

Forty seven control patients were studied. Multiple biopsies were obtained from the second part of the duodenum of 25 patients (mean age 52.1, range 24-78) at endoscopy. Jejunal tissue was obtained from 22

Address for correspondence: Dr J Crabtree, Department of Medicine, St James's University Hospital, Leeds LS9 7TF.

Accepted for publication 25 August 1988.

patients (mean age 37.9 years, range 16–64) undergoing examination for possible coeliac disease using a Crosby capsule or a Quinton hydraulic multiple biopsy instrument. The mucosa of control patients was normal at histological examination and no other abnormalities were demonstrated in these patients.

This project was approved by the Committee for Clinical Research (Ethics) of the Leeds Eastern Health Authority (1985).

Coeliac patients

Jejunal biopsies were obtained from 23 patients with coeliac disease. Ten were untreated (two men, eight women; mean age 38.1 years, range 22–53) on gluten containing diets at the time of biopsy. Five of the untreated patients had subtotal villous atrophy and five had severe partial villous atrophy on histological examination. Nine of the untreated patients have thus far shown histological improvement on gluten free diets. Jejunal biopsies were also obtained from one IgA deficient untreated coeliac patient (age 22) with subtotal villous atrophy. Jejunal biopsies were obtained from 12 treated coeliac patients (nine women, three men; mean age 32.9 years, range 17–43), who had been on gluten free diets for a minimum of six months (maximum 12 years). Biopsies from nine patients were histologically normal, while three showed mild partial villous atrophy.

ISOLATION OF INTESTINAL LYMPHOID CELLS

Biopsies were collected into culture medium consisting of RPMI 1640 additionally buffered with 20 mM HEPES (Flow Laboratories Ltd, Rickmansworth, Herts) and supplemented with 40 µg/ml Gentamycin and 10% heat inactivated fetal calf serum (FCS, Sera-Lab Ltd, Crawley) (complete medium) and processed within one hour. Cells were isolated by a modification of the methods of Bull and Bookman,¹¹ with the exception that no separate isolation of intraepithelial lymphocytes was undertaken. Briefly, mucus was removed from the biopsies by incubation for 15 minutes at 22°C in phosphate buffered saline (PBS) containing 1 mM dithiothreitol (Sigma Chemicals, Poole). After washing four times in PBS, the tissue was finely minced and incubated for three hours with agitation in complete medium with 125 U/ml collagenase (Type 1A, Sigma) at 37°C. The crude cell suspension was layered over FCS to allow sedimentation of debris¹⁵ before centrifugation over Lymphoprep (Nyegaard, Norway) at 800 g (15 minutes). The interface cells were collected, washed twice in RPMI 1640 and resuspended in complete medium. Viability of the lymphocytes was assessed by Trypan blue exclusion. The above cell isolation procedures does not affect the capacity of peripheral lymphocytes to secrete immunoglobulins *in vitro*.¹⁶

ISOLATION OF PERIPHERAL BLOOD

LYMPHOCYTES

Peripheral blood lymphocytes were isolated from heparinized venous blood, collected at the time of the investigative procedure, by centrifugation (450 g, 20 minutes) over Lymphoprep. Interface cells were washed twice with RPMI 1640 and T cell enriched fractions (PBT) were prepared by E-rosetting with 2-amino ethylisothiuronium bromide treated sheep red blood cells.¹⁷

IN VITRO IMMUNOGLOBULIN SECRETION

Intestinal lymphocytes were cultured at 2×10^6 cells/ml in 96 well round bottomed microtitre plates (Flow Laboratories) in a final volume of 200 µl. Cultures, set up in duplicate or where possible triplicate, were maintained in a 5% CO₂ humidified incubator at 37°C. After six days the culture supernatants were removed, centrifuged at 10000 g and stored at –70°C. Initial experiments showed that optimum immunoglobulin secretion occurred without the addition of pokeweed mitogen (Sigma, Poole) and mean intra-assay variability of cultures was 9.7%. The regulation of immunoglobulin secretion was examined by coculturing intestinal mucosal lymphocytes from some patients with autologous PBT at ratios of 1:1, 1:5, and 1:10. Control cultures of PBT alone, with and without pokeweed mitogen, were performed for each experiment. For measurement of performed intracellular immunoglobulins in mucosal lymphocytes, cells were frozen at 2×10^6 cells/ml for later sonication and assay.

MEASUREMENT OF IMMUNOGLOBULINS

The culture supernatants were assayed for total IgG, IgM, and IgA by enzyme linked immunoabsorbent assay (ELISA) as previously described.^{6,18} The amounts of secreted immunoglobulin were estimated from standard curves of human reference serum (Nyegaard) and the results expressed in µg/10⁶ cells. The detection limits for IgG, IgM, and IgA were 65, 43, and 21 ng/ml respectively.

IMMUNOFLUORESCENCE ANALYSIS OF CELL POPULATIONS

Air dried cytocentrifuged preparations (Shandon) of isolated mucosal lymphocytes and PBT were stored at –70°C until processed. The percentage Ig+ cells in the PBT preparations was assessed by direct immunofluorescence using fluorescein isothiocyanate (FITC) or tetraethyl rhodamine isothiocyanate (TRITC) conjugated antisera to human IgG, IgA, IgD, and IgM (Dako Ltd, High Wycombe, Bucks). The ratios of CD4:CD8 in cell isolates were determined by double label immunofluorescence as previously described² using the monoclonal anti-

bodies UCHT1 (CD3, IgG, Oxoid, Basingstoke) and RFT8 (CD8, IgM, Royal Free Hospital). Second layer goat antimouse Ig class specific antibodies were FITC conjugated anti-IgG and TRITC conjugated anti-IgM (Southern Biotechnology Associates Inc, USA). A minimum of 150 cells per isolate were examined on a Zeiss Standard microscope using epifluorescent illumination and selective filters for FITC and TRITC. The percentage CD4+ (helper/inducer) cells was assessed by estimating the number of CD3+ cells which were CD8-.

STATISTICAL ANALYSIS

Analysis of variance was carried out using the Kruskal-Wallis test. Statistical comparisons between mean values of unpaired data were carried out using the Mann-Whitney U test for non-parametric data. Analysis of paired data was carried out using the Wilcoxon's signed-rank test.

Results

CELL YIELD AND CHARACTERISATION

The yield of mucosal lymphoid cells from normal duodenal and jejunal biopsies was $1.1 (0.63) \times 10^6$ cells/mg wet weight. Biopsies from patients with treated and untreated coeliac disease yielded $1.18 (0.65) \times 10^6$ and $1.38 (0.91) \times 10^6$ cells/mg respectively. Viability of the isolated intestinal lymphocytes, as judged by Trypan blue exclusion, was greater than 95%.

The ratio of CD4:CD8 T lymphocytes in the mucosal isolates from control and coeliac patients is shown in Figure 1. The mean CD4:CD8 ratio of jejunal lymphocytes of untreated coeliac patients was significantly higher ($p < 0.05$) than that of control and treated coeliac patients. Peripheral blood T cell populations contained $< 1\%$ smlg+ cells. There was no significant difference between the mean CD4:CD8 ratios of PBT from control and coeliac patients (treated and untreated), which were 1.99:1 (0.72) ($n=18$) and 1.81:1 (0.61) ($n=12$) respectively.

IMMUNOGLOBULIN SECRETION BY INTESTINAL LYMPHOCYTES

Control patients

The concentration of preformed immunoglobulins in the duodenal lymphocytes of six control patients was very low (mean values in μg per 10^6 cells were IgA < 0.3 ; IgM < 0.2 ; IgG = 0). The amounts of IgA and IgM secreted spontaneously *in vitro* during six days culture is shown in Figures 2 and 3. IgA was the predominant immunoglobulin secreted by both duodenal and jejunal mucosal cells. The median amount of IgA produced by jejunal lymphocytes was

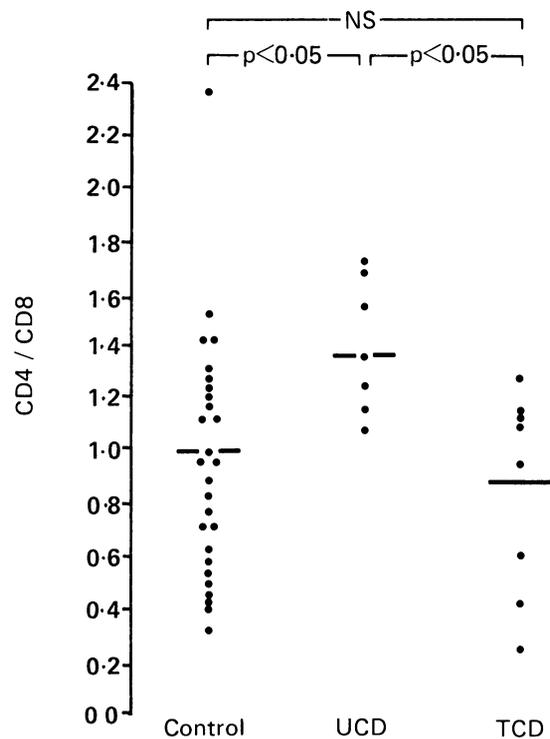


Fig. 1 CD4:CD8 ratio of intestinal lymphocytes isolated from control patients (duodenal and jejunal) and untreated (UCD) and treated coeliac (TCD) patients (jejunal).

approximately half that of the duodenal cells. The jejunal cells secreted significantly lower quantities of IgM ($p < 0.05$) than the duodenal cells. No quantifiable IgG was secreted by jejunal lymphocytes and only very low levels were detected in duodenal culture supernatants ($0.23 (0.07)$ per 10^6 cells). There was no correlation between the CD4:CD8 ratio of the mucosal lymphocytes and the *in vitro* secretion of IgM or IgA.

Coeliac patients

Isolated jejunal lymphocytes of patients with untreated coeliac disease secreted significantly higher amounts of IgA ($p < 0.05$) and IgM ($p < 0.001$) during six days culture *in vitro* than jejunal lymphocytes from control patients (Figs 2, 3). The IgM secretion was also significantly higher ($p < 0.01$) than that of duodenal lymphocytes from normal patients. In contrast with the controls, in six of the 10 untreated coeliac patients, IgM was the predominant immunoglobulin secreted. The IgA secretion did not differ significantly from the duodenal control values. The median values for IgA and IgM secretion in the untreated coeliac patients were 2.9 and 5.5 times those of the jejunal controls. The IgA deficient

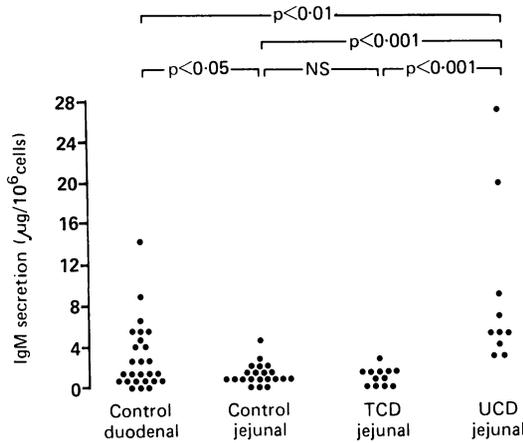


Fig. 2 Spontaneous IgM secretion in vitro during six days by intestinal lymphocytes from untreated (UCD) and treated (TCD) coeliac and control patients. Kruskal-Wallis analysis of variance ($p < 0.001$).

untreated coeliac patient secreted higher quantities of IgM ($38 \mu\text{g}/10^6$ cells) than the patients with no IgA deficiency. Significant IgG secretion was detected in only four untreated coeliacs (values 2.75 [IgA deficient], 1.25 , 1.19 , and $0.48 \mu\text{g}/10^6$ cells).

In treated coeliac patients, the secretion of IgA and IgM by isolated lymphocytes was significantly less ($p < 0.001$) than that of untreated patients. The treated values did not differ significantly from those of jejunal cells from control patients.

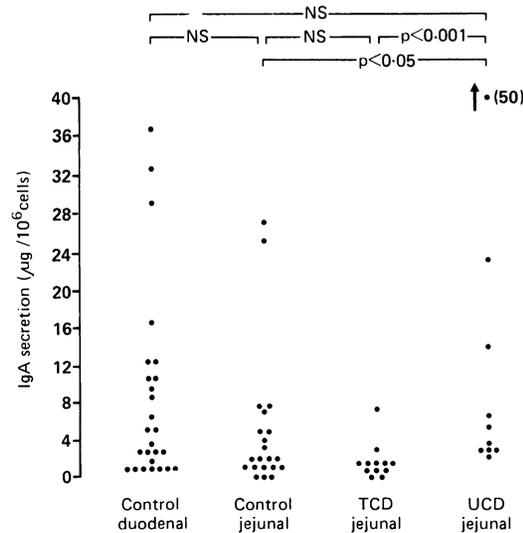


Fig. 3 Spontaneous IgA secretion in vitro during six days by intestinal lymphocytes from untreated (UCD) and treated (TCD) and control patients. Kruskal-Wallis analysis of variance ($p < 0.001$).

EFFECTS OF PERIPHERAL T CELLS ON IMMUNOGLOBULIN SECRETION

Control patients

A dose dependent increase in both IgA and IgM secretion by duodenal lymphocytes of control patients occurred on coculture with increasing concentrations of autologous peripheral T lymphocytes (Table). Significant increases were evident for both isotypes at ratios of 1:5 and 1:10 (IgM $p < 0.05$; IgA $p < 0.01$ by paired Wilcoxon's rank-test). Control cultures of PBT with and without pokeweed mitogen resulted in no measurable immunoglobulin secretion. Coculturing jejunal lymphocytes from control patients with autologous PBT had no significant effect on immunoglobulin secretion at any cell ratio (Table, Figs 4, 5), and suppression of IgA secretion occurred in two patients with high spontaneous IgA secretion (Fig. 5).

Coeliac patients

The IgA and IgM secretion of jejunal lymphocytes from untreated coeliac patients was significantly increased ($p < 0.05$) by coculture with autologous peripheral blood T lymphocytes (Figs 4, 5). In treated coeliac patients no increase in IgM or IgA secretion on coculture with PBT was observed. Two coeliac patients, examined before and after gluten exclusion, showed a reduction in spontaneous immunoglobulin secretion and T-cell helper effects after treatment.

Discussion

The immunoregulatory control of humoral responses in the human intestine is not well understood. Local secretion will vary according to the number and

Table The effect of peripheral blood T lymphocytes on the IgM and IgA secretion by duodenal and jejunal lymphocytes of control patients

Ratio of mucosal cells to PBT	Immunoglobulin secretion mean (SE) ($\mu\text{g}/10^6$ cells)	
	IgM	IgA
	Duodenum (n=13)	
1:0	3.98 (0.69)	8.86 (2.4)
1:1	4.79 (1.01)	12.63 (3.5)
1:5	*5.70 (1.12)	**20.52 (6.6)
1:10	*6.98 (1.7)	**22.70 (6.2)
	Jejunum (n=9)	
1:0	1.74 (0.45)	7.59 (3.54)
1:5	1.24 (0.38)	5.32 (1.66)
1:10	2.19 (0.85)	6.44 (2.06)

Significance from 1:0, $p < 0.05$, ** $p < 0.01$, by Wilcoxon's paired ranking test.

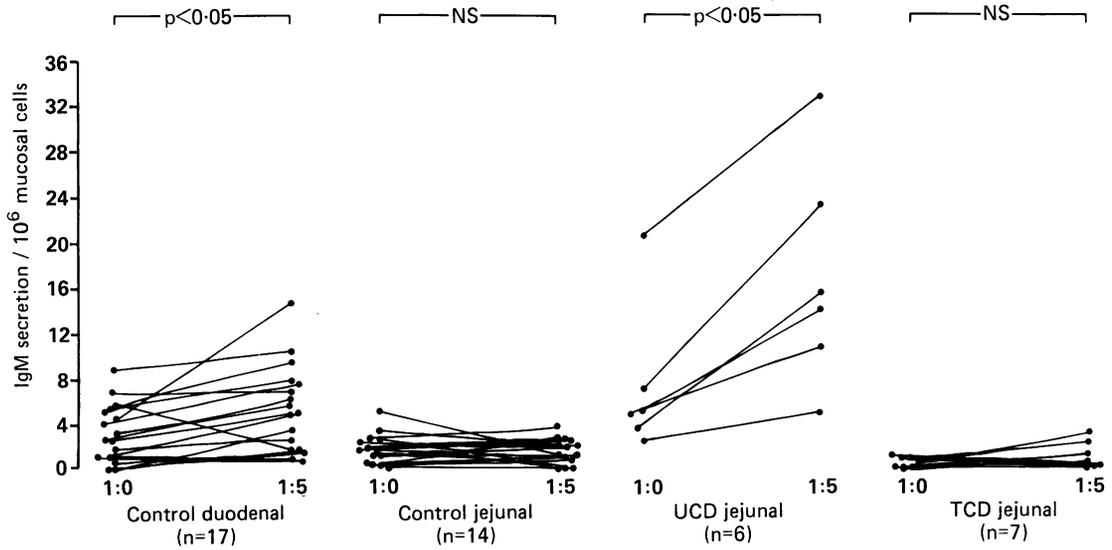


Fig. 4 Effect of autologous peripheral blood T lymphocytes on the secretion of IgM by intestinal lymphocytes from untreated (UCD) and treated (TCD) coeliac and control patients. Ratio of mucosal cells to PBT 1:5.

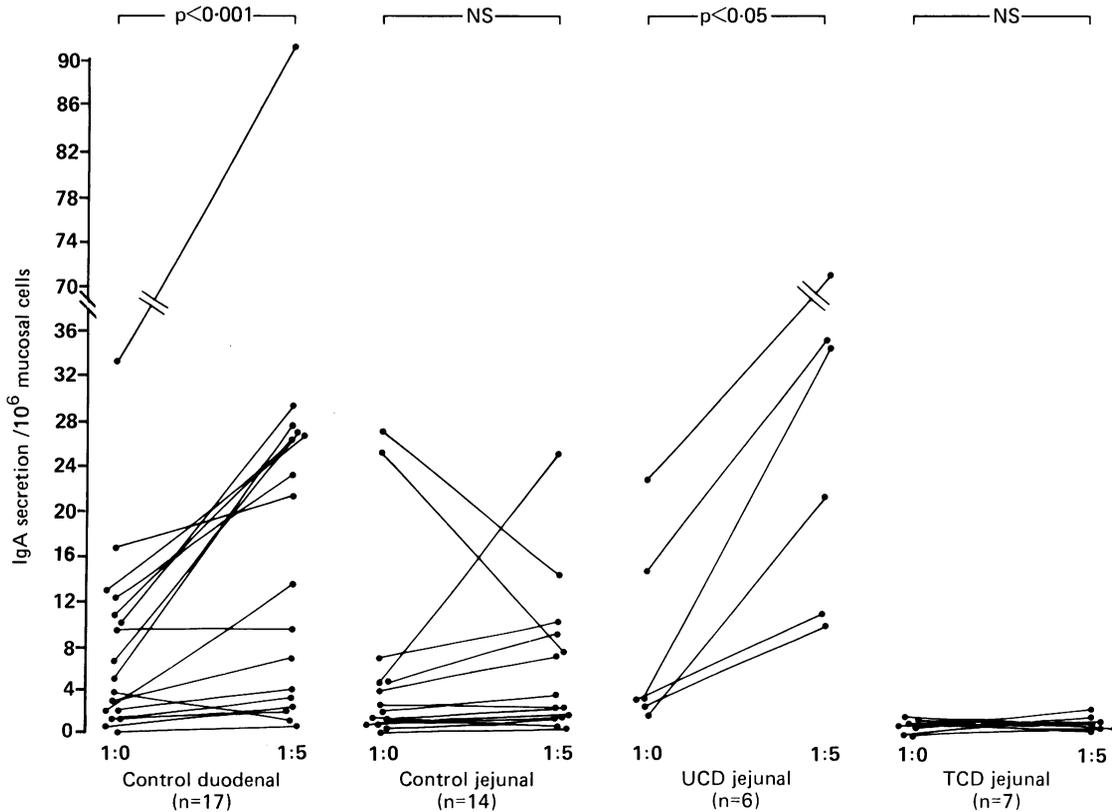


Fig. 5 Effect of autologous peripheral blood T lymphocytes on the secretion of IgA by intestinal lymphocytes from untreated (UTC) and treated (TCD) and control patients. Ratio of mucosal cells to PBT 1:5.

functional activity of mucosal immunoglobulin containing cells. The role of T-cells in regulating humoral responses however is complex with secreted lymphokines such as B-cell growth factor and B-cell differentiation factors affecting both the proliferation and the terminal differentiation of B lymphocytes into immunoglobulin secreting cells.¹⁹

In common with studies on isolated colonic and ileal human intestinal lymphocytes,²⁰⁻²² the results of this study show that lymphocytes from normal duodenal and jejunal mucosa have the capacity to spontaneously secrete IgA and IgM *in vitro*. The amounts of immunoglobulins spontaneously secreted by duodenal and jejunal lymphocytes differed, with lower levels of IgM being evident in the jejunum. Reduced numbers of IgM plasma cells are evident distally in the intestinal tract,²³ which might account for the observed differences in patients with normal mucosa. Although the isotype distribution of immunoglobulin-containing cells in the lamina propria is not uniform from the muscularis mucosae to the sub-epithelial region,⁵ a different distribution of IgM cells at the two locations would appear unlikely. Such factors, however, should be considered when studying the functional properties of isolated mucosal cell populations.

Significant increases were apparent in the amount of IgM and IgA spontaneously secreted by mucosal lymphocytes of patients with untreated coeliac disease. The high levels of spontaneous mucosal IgM secretion contrast with the reduced serum IgM levels observed in untreated coeliac patients,²⁴ suggesting a mucosal sequestration of sIgM-B cells rather than a specific defect in IgM production. Although the frequency of mucosal IgG plasma cells increases in coeliac disease,⁵ only minimal levels of IgG secretion were observed in this study. This confirms and extends the observations of Wood *et al*⁶ in which a rapid decrease in IgG secretion from jejunal tissue was observed after 24 hours culture *in vitro*. The increase in spontaneous secretion of IgM and IgA in untreated coeliac patients observed in this study parallels reported alterations in plasma cell isotype frequency,⁷ with the density of IgM plasma cells being increased relative to IgA cells.

Detailed phenotypic analysis of the isolated mucosal cells in coeliac patients was restricted by limited tissue availability. The mean CD4:CD8 ratio, however, of isolated jejunal lymphocytes from patients with untreated coeliac disease examined was significantly higher than that of control and treated coeliac patients. There is little alteration in the CD4:CD8 ratio of lymphocytes within the epithelium or lamina propria in coeliac disease.² Intraepithelial lymphocytes are largely CD8+, however,²⁵ and their absolute number per unit area of muscularis mucosae

is reduced in untreated coeliac disease.²⁶ The higher CD4:CD8 ratio of the cell isolates in the untreated coeliac patients therefore is likely to reflect a reduced ratio of intraepithelial to lamina propria cells. A selective loss of CD8 cells during the cell isolation procedure²⁷ restricted to untreated coeliac patients would appear unlikely. In untreated coeliac mucosa a higher proportion of CD8 cells co-express CD5, the function of these cells, however, is unknown.²

Despite the high spontaneous *in vitro* immunoglobulin secretion by normal duodenal lymphocytes, the mucosal cells were sensitive to helper influences of autologous PBT, IgA and IgM secretion being augmented in a dose dependent manner in coculture experiments. IgA secretion was particularly responsive to T-cell help, and it has been shown previously in humans that T helper cells for IgA are located both in the blood and secondary lymphoid tissue.²⁸ In contrast with the helper effects on IgA secretion, helper effects for IgM were relatively small but significant. Although the T-cell effects observed in this study were not isotype specific, the presence of separate T-cell populations with isotype-specific influences on terminal differentiation cannot be excluded. The observed helper effects on duodenal immunoglobulin secretion might, however, result from a complex interaction between mucosal immunocytes and PBT including contrasuppression.^{29,30}

The variation in response of jejunal lymphocytes of control patients is enigmatic. Low numbers of IgM responder cells might preclude the demonstration of T-cell helper effects for this isotype, but differences in IgA responsiveness relative to the duodenal lymphocytes might reflect the normal *in vivo* activation level of mucosal B or T cell populations. Alternatively the non-responsiveness of jejunal lymphocytes of control patients might be a consequence of a higher level of suppressor cell activation in the isolated mucosal population.

The significant T-cell helper effects, particularly for IgM, observed in untreated coeliac patients contrast markedly with the results obtained with normal jejunal tissue. A defect in T-cell regulation of intestinal humoral responses might account for local plasma cell infiltration.⁷ Alternatively, the changes might reflect altered cell populations secondary to tissue damage. Although no changes in the CD4:CD8 ratio of peripheral lymphocytes, as determined by monoclonal markers, is evident in coeliac disease,³¹ other studies have emphasised the heterogeneity of human regulatory T cell populations.³² Enhanced mucosal immunoglobulin synthesis could result from failure of suppressor cell function or increased contrasuppressor activity.³³

The observed perturbations in humoral mucosal responses in untreated coeliac patients may therefore

reflect changes in regulatory T cell populations. With increasing characterisation of human T cell subsets, greater understanding of the immunoregulatory function of T cells in humoral responses in coeliac patients should be possible.

This work was supported by the Medical Research Council. We would like to thank Dr J Findlay, and the staff of the Gastroenterology Departments of Bradford Royal Infirmary and St James's University Hospital for their cooperation and Dr L K Trejdosiewicz for valuable discussions.

References

- 1 Strober W. Gluten-sensitive enteropathy, an abnormal immunologic response of the gastrointestinal tract to a dietary protein. In: Shortner RG, Kirsner JB, eds. *Gastrointestinal immunity for the clinician*. Orlando: Grove & Stratton Inc, 1985: 75–112.
- 2 Malizia G, Trejdosiewicz LK, Wood GM, Howdle PD, Janossy G, Losowsky MS. The microenvironment of coeliac disease: T cell phenotypes and expression of the T2 'T blast' antigen by small bowel lymphocytes. *Clin Exp Immunol* 1985; **60**: 437–46.
- 3 Kelly J, O'Farrelly C, O'Mahoney C, Weir DG, Feighery C. Immunoperoxidase demonstration of the cellular composition of the normal and coeliac small bowel. *Clin Exp Immunol* 1987; **68**: 177–88.
- 4 Lancaster-Smith M, Kumar P, Marks R, Clark ML, Dawson AM. Jejunal mucosal immunoglobulin-containing cells and jejunal fluid immunoglobulins in adult coeliac disease and dermatitis herpetiformis. *Gut* 1974; **15**: 371–6.
- 5 Bakelien K, Brandtzaeg P, Fausa O. Immunoglobulins in jejunal mucosa and serum from patients with adult coeliac disease. *Scand J Gastroenterol* 1977; **12**: 149–59.
- 6 Wood GM, Howdle PD, Trejdosiewicz LK, Losowsky MS. Jejunal plasma cells and in vitro immunoglobulin production in adult coeliac disease. *Clin Exp Immunol* 1987; **69**: 123–32.
- 7 Dhesi I, Marsh MN, Kelly C, Crowe P. Morphometric analysis of small intestinal mucosa. 2. Determination of lamina propria volumes: plasma cell and neutrophil populations within control and coeliac disease mucosae. *Virchows Arch [Pathol Anat]* 1984; **403**: 173–80.
- 8 Fluge G, Aksnes L. Quantification of immunoglobulins after organ culture of human duodenal mucosa. *J Pediatr Gastroenterol Nutr* 1983; **2**: 62–70.
- 9 Wood GM, Shires S, Howdle PD, Losowsky MS. Immunoglobulin production by coeliac biopsies in organ culture. *Gut* 1986; **27**: 1151–60.
- 10 Scott BB, Scott DG, Losowsky MS. Jejunal mucosal immunoglobulins and complement in untreated coeliac disease. *J Pathol* 1977; **121**: 219–23.
- 11 Bull DM, Bookman MA. Isolation and functional characterisation of human intestinal mucosal lymphoid cells. *J Clin Invest* 1977; **59**: 966–74.
- 12 Crofton RW, Cochran C, McClelland DBL. Preparation of lymphoid cells from small specimens of human gastrointestinal mucosa. *Gut* 1978; **19**: 898–906.
- 13 Goodacre R, Davidson R, Singal D, Bienenstock J. Morphologic and functional characteristics of human intestinal lymphoid cells isolated by a mechanical technique. *Gastroenterology* 1979; **76**: 300–8.
- 14 Elson CO, Weiserbs DB, Ealding W, Machelski E. T-helper cell activity in intestinal lamina propria. *Ann NY Acad Sci* 1983; **409**: 230–7.
- 15 Shortman K, Williams N, Adams P. The separation of different cell classes from lymphoid organs. 5. Simple procedures for the removal of cell debris, damaged cells and erythroid cells from lymphoid cell suspensions. *J Immunol Methods* 1972; **1**: 273–87.
- 16 Smart CJ, Trejdosiewicz LK, Badr-el-Din S, Heatley RV. T lymphocytes of the human colonic mucosa: functional and phenotypic analysis. *Clin Exp Immunol* 1988; **73**: 63–9.
- 17 Jondall M, Holm G, Wigzell H. Surface markers on human T and B lymphocytes. 1. A large population of lymphocytes forming nonimmune rosettes with sheep red blood cells. *J Exp Med* 1972; **136**: 207–15.
- 18 Wood GM, Trejdosiewicz LK, Losowsky MS. ELISA for measurement of secretory IgA distinct from monomeric IgA. *J Immunol Methods* 1987b; **97**: 269–74.
- 19 Jelinik DJ, Lipsky PJ. Regulation of human B lymphocyte activation, proliferation, and differentiation. *Adv Immunol* 1987; **40**: 1–59.
- 20 MacDermott RP, Nash GS, Bertovich MJ, Seiden MV, Bragdon MJ, Beale MG. Alterations of IgM, IgG, and IgA synthesis and secretion by peripheral blood and intestinal mononuclear cells from patients with ulcerative and Crohn's disease. *Gastroenterology* 1981; **81**: 844–52.
- 21 MacDermott RP, Nash GS, Bertovich MJ, *et al*. Altered patterns of secretion of monomeric IgA and IgA subclass 1 by intestinal mononuclear cells in inflammatory bowel disease. *Gastroenterology* 1986; **91**: 379–85.
- 22 Danis VA, Heatley RV. Evidence for regulation of human colonic mucosal immunoglobulin secretion by intestinal lymphoid cells. *J Clin Lab Immunol* 1987; **22**: 7–11.
- 23 Brandtzaeg P. The B cell system. In: Brostoff J, Challacombe SJ, eds. *Food allergy and intolerance*. Sussex: Baillière Tindall, 1987: 118–55.
- 24 Hobbs JR, Hepner CW. Deficiency of M-globulin in coeliac disease. *Lancet* 1968; **i**: 217–20.
- 25 Selby WS, Janossy G, Jewell DP. Immunohistological characterisation of intraepithelial lymphocytes of the human gastrointestinal tract. *Gut* 1981; **22**: 169–76.
- 26 Marsh MN. Coeliac disease. In: Marsh MN, ed. *Immunopathology of the small intestine*. Chichester: John Wiley, 1987: 371–99.
- 27 Selby WS, Janossy G, Bofill M, Jewell DP. Intestinal lymphocyte subpopulations in inflammatory bowel disease: an analysis by immunohistological and cell isolation techniques. *Gut* 1984; **25**: 32–40.
- 28 McCaughan GW, Adams E, Basten A. Human antigen-specific IgA responses in blood and secondary lymphoid tissue: an analysis of help and suppression. *J Immunol* 1984; **132**: 1190–6.
- 29 Gershon RK, Eardley DD, Durum S, *et al*. Contraception: a novel immunoregulatory activity. *J Exp Med* 1981; **153**: 1533–46.

- 30 Lehner T, Avery J, Jones T. Separation and characterization of a subset of human T8+ cells which function as antigen-presenting and contrasuppressor cells. *Immunology* 1985; **54**: 713–22.
- 31 Selby WS, Janossy G, Bofill M, Jewell DP. Lymphocyte subpopulations in the human small intestine. The findings in normal mucosa and in the mucosa of patients with adult coeliac disease. *Clin Exp Immunol* 1983; **52**: 219–28.
- 32 Thomas Y, Rogoziuski L, Chess L. Relationship between human T-cell functional heterogeneity and human T-cell surface molecules. *Immunol Rev* 1983; **74**: 113–28.
- 33 Green DR, Gold J, St Martin S, Gershon R, Gershon RK. Microenvironmental immunoregulation: possible role of contrasuppressor cells in maintaining immune response in gut-associated lymphoid tissues. *Proc Natl Acad Sci USA* 1982; **79**: 889–92.