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Gamma/delta T cells and the diagnosis of coeliac disease

J. SPENCER, P. G. ISAACSON, T. T. MACDONALD†, A. J. THOMAS* & J. A. WALKER-SMITH*
Department of Histopathology, University College and Middlesex School of Medicine, Bland Sutton Institute, London,
**Academic Unit of Paediatric Gastroenterology, Queen Elizabeth Hospital For Children, London, and*
†*St Bartholomew's Hospital, London, England*

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SUMMARY

Gamma/delta T cells are increased in the gut epithelium of patients with coeliac disease compared with normal controls. The aim of this study was to determine whether the increase in $\gamma\delta$ intraepithelial lymphocytes (IEL) is specific for coeliac disease, in which case it could be of diagnostic importance. Biopsies were obtained from children with no intestinal disease, coeliac disease, cow-milk-sensitive enteropathy/post-enteritis syndrome (CMSE/PES) and miscellaneous other enteropathies ($n=67$). Intraepithelial CD3$^+$ and $\gamma\delta$ T cells were identified in frozen sections using peroxidase immunohistochemistry. In normal biopsies there were 0–7 $\gamma\delta$ IEL/100 cells in the epithelium. In untreated coeliac patients this increased to 9–22 $\gamma\delta$ IEL/100 cells in the epithelium ($P=0{\cdot}000004$). Of 27 patients with morphologic intestinal damage which was not due to coeliac disease, four with CMSE/PES had $\gamma\delta$ IEL/100 cells in the epithelium in the same range as the patients with coeliac disease. Of these, two had high densities of CD3$^+$ IEL in the epithelium and were indistinguishable from patients with untreated coeliac disease. The other two could be excluded as possible coeliacs because their CD3$^+$ IEL/100 epithelial cells were in the normal range. Thus an increase in $\gamma\delta$ IEL is not specific for coeliac disease. However, enumeration of both of $\gamma\delta$ IEL and CD3$^+$ IEL densities will be useful in the exclusion of coeliac disease as a diagnosis in some children.

Keywords intraepithelial lymphocytes coeliac disease T cell receptors $\gamma\delta$ T cells

INTRODUCTION

The diagnosis of coeliac disease in childhood, as defined by the original ESPGAN criteria (Meeuwisse, 1970), is a time-consuming process. It involves an initial small intestinal biopsy, a second small intestinal biopsy following clinical remission on a gluten-free diet, and at least a third biopsy to show small intestinal mucosal damage after gluten challenge. Such damage may take many months to develop, so fourth and even fifth biopsies may be required to finally establish the diagnosis. Thus an alternative means of diagnosing coeliac disease would be of particular advantage to obviate the need for repeat invasive procedures in children. Definitive diagnosis of coeliac disease is of particular importance in children under 2 years of age because there are several other causes of small intestinal enteropathy apart from coeliac disease, including cow-milk-sensitive enteropathy, post-enteritis syndrome and a variety of infectious agents (Walker-Smith *et al.* 1990). Previously proposed methods of differentially diagnosing coeliac disease have included analysis of IgA anti-gliadin antibodies (Savilahti *et al.*, 1983), anti-reticulin (Unsworth, Walker-Smith & Holborow, 1983) and IgA anti-endomysium antibodies (Chozelski *et al.*, 1984), but these are not specific for coeliac disease. A recent proposal is that gluten challenge be made rectally, where it is easier to take biopsies (Loft, Marsh & Crowe, 1990). However, the effectiveness of using this technique to diagnose coeliac disease in children is not known.

The possibility of diagnosing coeliac disease by studying the intraepithelial lymphocyte (IEL) population was studied, and it was shown that mitotic figures are more commonly seen in the jejunal IEL of patients with coeliac disease than those with non-coeliac enteropathies (Marsh, 1982; Marsh & Haeney, 1983). However, this technique has failed to gain general acceptance. It has recently been shown by several groups that $\gamma\delta$ T cells are increased in frequency in the IEL population of patients with coeliac disease (Spencer *et al.*, 1989; Halstensen, Scott & Brandtzaeg, 1989; Savilahti, Arato & Verkasalo, 1990). It has been suggested that if the increase in $\gamma\delta$ T cells is specific for coeliac disease, this could be a significant aid to diagnosis (Walker-Smith *et al.*, 1990). Previous studies have compared coeliac disease with normal biopsies or inappropriate disease controls (e.g. inflammatory bowel disease) so that it has not been established whether enumeration of $\gamma\delta$ T cells would indeed be diagnostically useful.

Correspondence: Dr Jo Spencer, Department of Histopathology, University College and Middlesex School of Medicine, Bland Sutton Institute, Riding House St, London WC1P 7PN, UK.

The aim of this study was therefore to compare γδ T cells in the gut epithelium in a large series of biopsies from children in whom subsequent clinical diagnoses were made, to determine whether this would enable an early and reliable diagnosis of coeliac disease to be made using a single initial biopsy.

SUBJECT AND METHODS

Tissue collection and Processing

Small intestinal biopsies were obtained using a double-port paediatric Crosby capsule from patients with a clinical history suggestive of coeliac disease. Unless otherwise stated, patients were on normal diets at the time of biopsy. Half of each biopsy was fixed and processed for routine histology and half was snap frozen and stored in liquid nitrogen for study of surface markers.

Patients with coeliac disease (n = 12)

All patients in this group were diagnosed as having coeliac disease according to the revised ESPGAN criteria (Walker-Smith *et al.*, 1990). Eight were on normal diets and four were on gluten-free diets at the time of biopsy. The mean age of the patients on normal diets was 4 years and 7 months (range 1 year and 1 month–9 years and 11 months) and of patients on gluten-free diets, 4 years and 2 months (range 1 year and 5 months–8 years and 6 months).

Patients with histologically normal biopsies (n = 28).

Histologically, normality was defined in paraffin sections as villus:crypt ratio of 3:1 or more, and no abnormality of the brush border at the villous tips. Ten patients were diagnosed as having toddlers' diarrhoea, four post-enteritis syndrome (resolved), 10 failure to thrive (unknown cause), one multiple food allergy, one egg allergy, one lactose intolerance and one ileal stenosis. The mean age of the patients was 2 years and 10 months (range 4 months–13 years).

Patients with cow-milk-sensitive enteropathy and post-enteritis syndrome (CMSE/PES) (n = 18)

Fifteen of these biopsies showed partial villous atrophy, two showed patchy partial villous atrophy, and one showed severe villous atrophy. It was impossible to separate the patients in this group into those sensitive to cows' milk and those with PES because both conditions may be transient and indeed may co-exist, precluding accurate clinical diagnosis (Walker-Smith, 1988). All were treated with and responded to a cow-milk-free diet but in none were serial biopsies related to milk elimination or challenge performed (Walker-Smith *et al.*, 1978). Cryptosporidia were isolated from the stools and biopsies of two of these patients. The mean age of patients in this group was 10 months (range 3 months–4 years and 7 months).

Patients with miscellaneous idiopathic enteropathies (n = 9)

Six biopsies showed partial villous atrophy and three biopsies showed patchy partial villous atrophy. Two patients were diagnosed as having idiopathic colitis, three idiopathic enteropathy, one intractable diarrhoea of infancy, one eosinophilic gastroenteritis, one failure to thrive (unknown cause), one gluten allergy (Rudd, Manuel & Walker-Smith. 1981). Mean age was 3 years and 1 month (range 3 months–12 years and 6 months).

Immunocytochemistry

Immunohistological studies were carried out on 8 μm acetone-fixed sections of snap-frozen jejunal biopsies. The following primary antibodies were used: CD3 (Dako, High Wycombe, UK) which is expressed on all T cells and TCRδ1 (T Cell Sciences, Cambridge, MA) which recognizes all γδ T cells. Following incubation in primary antibody, peroxidase-conjugated rabbit anti-mouse immunoglobulin was applied to the sections and the peroxidase activity was visualized using diamino benzidine substrate.

Quantification of IEL

The density of cells expressing CD3 or TCRδ1 in the epithelium was determined by counting the number of stained cells as a percentage of the total cells in the epithelium, both lymphoid and epithelial. Counts were made on serial sections, in triplicate, using ×400 total magnification. IEL per 100 total cells in the epithelium were quantified, because in frozen sections it is not always possible to tell whether a negative cell is epithelial or lymphoid. Only cells in the surface epithelium were counted. Based on the densities of $CD3^+$ IEL and γδ IEL/100 cells in the epithelium, the frequency of γδ IEL in the CD3 population was deduced.

Statistical analysis

Data from all groups were tested to determine whether they conformed to samples taken from a normally distributed population. Data from the group of normal biopsies were not normally distributed and thus statistical comparisons were made using the Mann–Whitney *U*-test.

RESULTS

The density of $CD3^+$ IEL in each patient was determined (Fig. 1a). As expected, patients with coeliac disease had a higher density of $CD3^+$ IEL than any other group (coeliac disease *versus* normal, $P = 4{\cdot}3 \times 10^{-5}$; coeliac disease *versus* CMSE/PES, $P = 1{\cdot}9 \times 10^{-4}$; coeliac disease *versus* miscellanous biopsies $P = 3{\cdot}8 \times 10^{-4}$). However, there was some overlap in that four of the normal patients and four of the patients with CMSE/PES had $CD3^+$ IEL densities within the range seen in the coeliac patients.

The density of γδ IEL was next determined (Fig. 1b). The density of γδ IEL was increased in untreated coeliac patients compared with all other groups (coeliac disease *versus* normal, $P = 4{\cdot}1 \times 10^{-6}$; coeliac disease *versus* CMSE/PES, $P = 4{\cdot}3 \times 10^{-4}$; coeliac disease *versus* miscellaneous enteropathies, $P = 2{\cdot}7 \times 10^{-4}$). There was no overlap between either the normal biopsies or the miscellaneous enteropathies with the untreated coeliacs. However, four of the 18 patients with CMSE/PES were in the range of the treated coeliacs and details of the $CD3^+$ and γδ IEL densities, and the frequency of γδ $CD3^+$ IEL of these four patients are shown in Table 1. In the two with the highest values, $CD3^+$ IEL were also high, thus these were indistinguishable from untreated coeliac disease. In the other two with only slightly raised values, the $CD3^+$ IEL density was normal thus excluding coeliac disease.

Four patients with treated coeliac disease were studied. In the two with the highest densities of $CD3^+$ IEL, the density of γδ IEL was in the range seen in untreated coeliacs. In the two with the lowest densities of $CD3^+$ IEL the density of γδ IEL was in the range seen in the normal biopsies (Fig. 2).

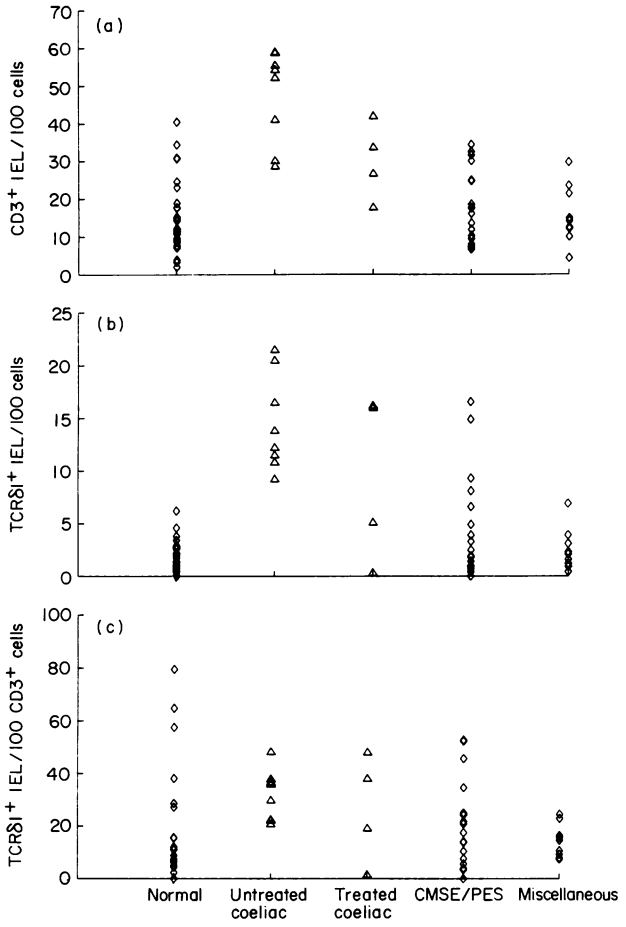

Fig. 1. Scatter-plots showing (a) CD3+ intra-epithelial lymphocytes (IEL)/100 cells in the epithelium; (b) TCRδ1+ IEL/100 cells in the epithelium; and (c) TCRδ1+ IEL/100 CD3+ cells in the epithelium, in normal biopsies ($n=28$), biopsies from untreated coeliacs ($n=8$), treated coeliacs ($n=4$), cow-milk-sensitive enteropathies and/or post-enteritis syndrome (CMSE/PES) ($n=18$), and miscellaneous other enteropathies ($n=9$).

Table 1. Densities of CD3+ and TCRδ1+ intra-epithelial lymphocytes (IEL) and the frequency of CD3+ IEL expressing TCRδ1 in four patients with cow-milk-sensitive enteropathy/post-enteritis syndrome (CMSE/PES) with γδ T cell densities within the range seen in coeliac disease

Patient with CMSE/PES	CD3+ IEL/ 100 cells in epithelium	TCRδ1+ IEL/ 100 cells in epithelium	TCRδ1+/ 100 CD3+ IEL
1	17·6	9·3	52·8
2	17·7	8·1	45·8
3	31·7	16·6	52·4
4	32·6	14·9	45·7

The frequency of CD3+ IEL expressing γδ was then deduced (Fig. 1c). The frequency in untreated coeliac disease was increased compared with normal biopsies ($P=0·002$), CMSE/PES ($P=0·047$) and miscellaneous enteropathies ($P=0·002$). Patients with CMSE/PES had a higher frequency of CD3+ IEL expressing γδ than did normal controls ($P=0·03$). It was also noticeable that this measurement did not distinguish between coeliacs and normals since seven of the normal biopsies had a frequency of γδ IEL as high or even higher than the untreated coeliacs. These patients, however, had a low densities of both CD3+ IEL/100 cells in the epithelium and γδ IEL/100 cells in the epithelium, and normal mucosal morphology. By deduction, therefore, these patients must be relatively lacking in IEL expressing αβ T cell receptor. Eight of the patients with CMSE/PES also had frequencies of γδ IEL/100 CD3+ cells comparable to untreated coeliacs.

DISCUSSION

This study shows, as we and others have shown previously, that there is an increase in γδ IEL in small intestinal biopsies of patients with untreated coeliac disease compared with patients with normal biopsies (Spencer *et al.*, 1989; Halstensen *et al.*, 1989; Savilhati *et al.*, 1990). This is apparent both in terms of density of γδ IEL in the epithelium and in the frequency of γδ IEL in the CD3+ IEL population. We now show that γδ IEL are also increased in the biopsies of some patients with CMSE/PES, which also have high densities of IEL and which histologically most closely resemble coeliac disease. Of the 27 patients studied with CMSE/PES or miscellaneous other enteropathies, all of whom had various degrees of histological abnormality, two in the CMSE/PES group had an increased density of CD3+ IEL, an increased density of γδ IEL, and a high frequency of γδ IEL in the CD3+ IEL population. Both of these biopsies were thus indistinguishable from biopsies from patients with coeliac disease. One of these patients had cryptosporidiosis and the other improved on a cow-milk-free diet. Since CMSE/PES is the most important differential diagnosis of coeliac disease in children, the failure of γδ T cells to distinguish these children from true coeliacs is disappointing. Nevertheless, the fact that 25 out of 27 patients with morphologic intestinal damage could be excluded as coeliacs demonstrates that γδ T cells can be used to exclude coeliac disease in most cases.

Other investigators have reported that patients with coeliac disease on gluten-free diets retain a high density of γδ IEL (Halstensen *et al.*, 1989; Savilhati *et al.*, 1990). Of the patients with coeliac disease on gluten-free diets in this study, two retained a high density of γδ IEL. However, these patients also had high densities of CD3+ IEL/100 cells in the epithelium. The two coeliac patients on gluten-free diets in this study with IEL densities within normal range also had normal densities of γδ IEL. We therefore propose that if the IEL density of a coeliac patient decreases, then the γδ IEL density also decreases. Using double immunofluorescence, Halstensen *et al.* (1990) showed directly that the population of CD3+ IEL expressing γδ increases in coeliac disease. The increase in γδ T cells in coeliac disease exceeds the increase in αβ T cells so that the increase in γδ IEL observed in coeliac disease is disproportionate and does not merely reflect an increase in the density of IEL. The reasons for this are unknown.

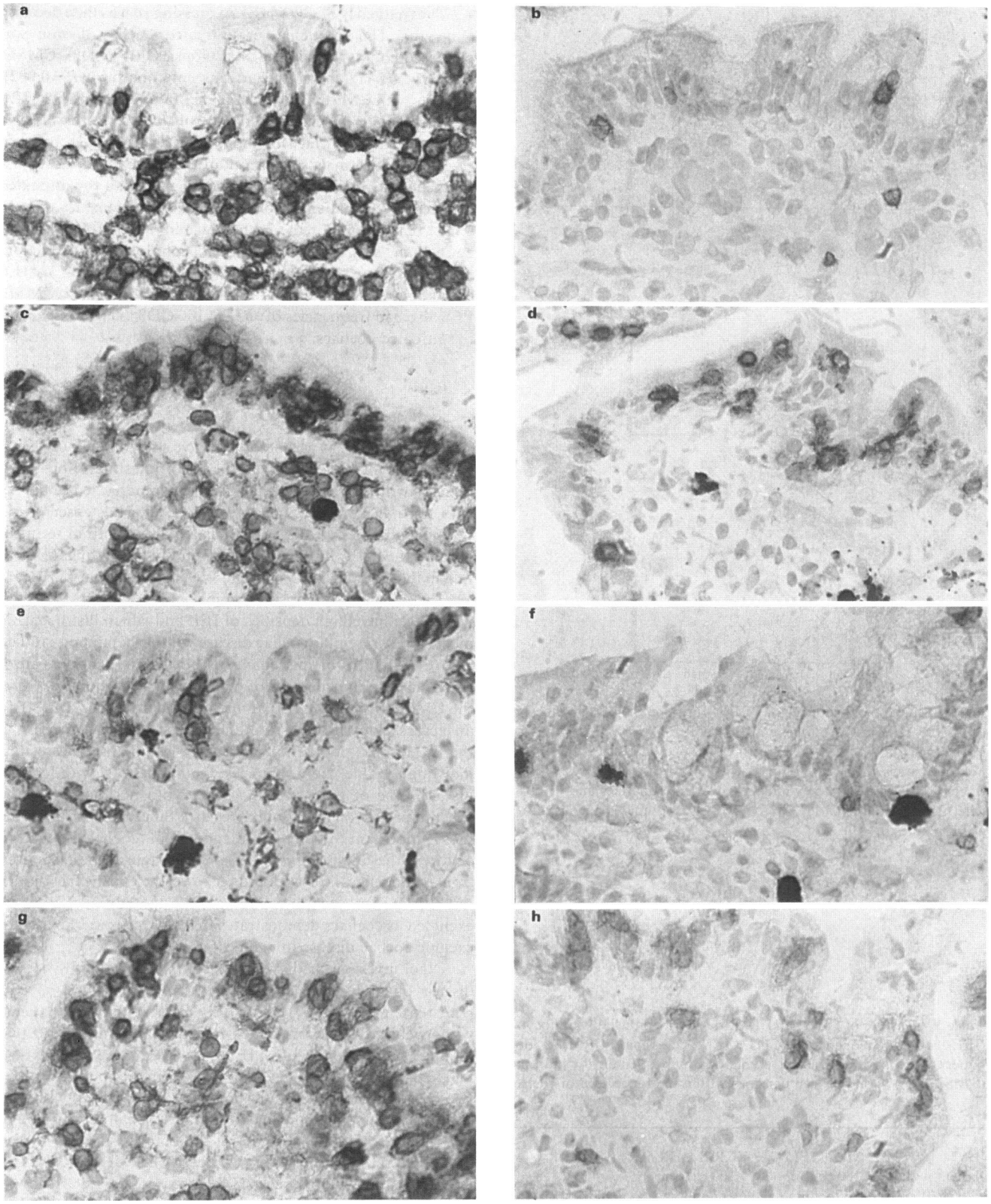

Fig. 2. Photomicrographs of normal small intestine (a, b), untreated coeliac small intestine (c, d), treated coeliac small intestine (e, f) and cow-milk-sensitive enteropathies and/or post-enteritis syndrome (CMSE/PES) small intestine with a high density of $CD3^+$ and $TCR\delta1^+$ IEL (g, h), stained with CD3 (a, c, e, g) and $TCR\delta1$ (b, d, f, h). IEL expressing $TCR\delta1$ are increased in density and in frequency within the $CD3^+$ IEL population in both coeliac disease and the case of CMSE/PES illustrated. The case of treated coeliac disease illustrated has normal levels of $CD3^+$ and $TCR\delta1^+$ IEL. Sections c and d and e and f are from biopsies taken from the same patient on normal and gluten-free diet, respectively. Immunoperoxidase staining; magnification × 160.

Enumeration of $\gamma\delta^+$ and $CD3^+$ IEL in a single initial biopsy will be of use in the diagnosis of coeliac disease. A low density of $\gamma\delta$ IEL in a patient with a high $CD3^+$ IEL density will enable coeliac disease to be excluded. A high density of $\gamma\delta$ IEL will not allow a positive diagnosis of coeliac disease to be made, however, as this is occasionally seen in other enteropathies.

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REFERENCES

CHOZELSKI, T.P., BEUTNER, D.H., SULEJ, J., TCHORZEWSKA, H., JABLONSKA, S., KUMAR, V. & KAPUSCINSKA, A., (1984) IgA anti-endomysium antibody. *Br. J. Dermatol.* **111,** 395.

HALSTENSEN, T.S., SCOTT, H. & BRANDTZAEG, P. (1989) Intraepithelial T cells of the TcR $\gamma\delta+$, CD8− and Vd1/Jd1+ phenotypes are increased in coeliac disaease. *Scand. J. Immunol.* **30,** 665.

LOFT, D.E., MARSH, M.N., & CROWE, P.T. (1990) Rectal gluten challenge and the diagnosis of coeliac disease. *Lancet,* **i,** 1293.

MARSH, M.N. (1982) Studies of intestinal lymphoid tissue. IV. The predictive value of raised mitotic indices among jejunal epithelial lymphocytes in the diagnosis of gluten sensitive enteropathy. *J. clin. Pathol.* **35,** 517.

MARSH, M.N. & HAENEY, M.N. (1983) Studies of intestinal lymphoid tissue. VI. Proliferative response of small intestinal epithelial lymphocytes distinguishes gluten-from non-gluten-induced entropathy. *J. clin. Pathol.* **36,** 149.

MEEUWISSE, G.W. (1970) Diagnostic criteria in coeliac disease. *Acta paediatr. Scand.* **59,** 461.

RUDD, P. MANUEL, P. & WALKER-SMITH, J. (1981) Anaphylactic shock in an infant after feeding with a wheat rusk. A transient phenomenon. *Postgrad. med. J.* **57,** 794.

SAVILAHTI, E., ARATA, A. & VERKASALO, M. (1990) Increased numbers of gamma/delta bearing lymphocytes in the epithelium of coeliac patients. In *Advances in Mucosal Immunology* (Ed. by T. T. MacDonald, S. J. Challacombe, P. W. Bland, C. R. Stokes, R. V. Heatley & A. McI. Mowat) p.61. Kluwer, Dordrecht.

SAVILAHTI, E., VIANDER, M., PERKKIO, M., VIANIO, E., KALIMO, K. & REUNALA, T. (1983) IgA gliadin antibodies: a marker of mucosal damage in childhood coeliac disease. *Lancet,* **i,** 320.

SPENCER, J., ISAACSON, P.G., DISS, T.C. & MACDONALD, T.T. (1989) Expression of disulphide linked and non-disulphide linked forms of the T cell receptor gamma/delta heterodimer in human intestinal intraepithelial lymphocytes. *Eur. J. Immunol.* **19,** 1335.

UNSWORTH, D.J., WALKER-SMITH, J.A. & HOLBOROW, E.J. (1983) Gliadin and reticulin antibodies in childhood coeliac disease. *Lancet,* **i,** 874.

WALKER-SMITH, J.A. (1988) *Diseases of the Small Intestine in Childhood* 3rd edn. Butterworth, London.

WALKER-SMITH, J.A., GUANDALINI, S., SCHMITZ, J., SCHMERLING, D.H. & VISAKORPI, J.K. (1990) Revised criteria for the diagnosis of coeliac disease. *Arch. Dis. Child. 65,* 909.

WALKER-SMITH, J.A., HARRISON, M., KILBY, A., PHILLIPS, A.D. & FRANCE, N. (1978) Cows milk sensitive enteropathy. *Arch. Dis. Child.* **53,** 375.