

# Intestinal antibody pattern of coeliac disease: association with $\gamma/\delta$ T cell receptor expression by intraepithelial lymphocytes, and other indices of potential coeliac disease

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## Abstract

**Patients with coeliac disease have IgM antibodies and IgA anti-gliadin antibody in gut secretions, and also high counts of intraepithelial lymphocytes (IEL) that express the  $\gamma/\delta$  T cell receptor. These features of intestinal immunity may be markers of latent coeliac disease. Their occurrence was examined in 77 patients referred for jejunal biopsy, in whom biopsy histology was normal, to establish the extent to which these, and other candidate markers (high total IEL count, serum IgA anti-gliadin antibody (AGA), and increased permeability) coexist in the same patient. Twelve patients had high serum anti-gliadin antibody titres and nine increased permeability. The  $\gamma/\delta$  IEL count was high ( $>5.5$  per mm villus epithelium) in nine patients, the intestinal antibody pattern was positive in 21, and the total IEL count was high ( $>40$  per 100 enterocytes) in 13. Overall, 31 patients had positive indices, but in 19 only a single test was abnormal. High  $\gamma/\delta$  IEL counts were found in six of 21 intestinal antibody positive patients, but in only two of 56 who were intestinal antibody negative ( $p < 0.001$ ); there were no other significant associations. Clinical tests of gluten sensitivity will be required to establish the prevalence of latent gluten sensitive enteropathy in the 40% of patients referred for jejunal biopsy in whom one or more of the immunological indices of potential coeliac disease is present.**

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Coeliac disease, defined as a permanent intolerance of the small bowel mucosa to gluten, is heterogeneous in its clinical presentation and pathological expression. There are also patients with 'latent coeliac disease', who have normal jejunal histology when eating a normal diet but at some other time, before or since, have been shown to have typical, severe gluten sensitive enteropathy.<sup>1</sup> Diagnostic criteria of latent coeliac disease are stringent, and the diagnosis is usually made in retrospect or by chance. We have therefore suggested that the term 'potential coeliac disease' is more appropriate in clinical practice, to be used while dietary manipulations are being undertaken in patients suspected of having latent coeliac disease.<sup>2</sup> If the findings are positive, the diagnosis of low grade pathology coeliac disease is then confirmed.

Recent studies have shown that a number of

intestinal immune features may be candidate markers of latent or potential coeliac disease:

(1) High counts of T cell receptor  $\gamma/\delta$  intraepithelial lymphocytes (IEL) have been shown in dermatitis herpetiformis and in a case of latent coeliac disease<sup>3,4</sup>;

(2) A coeliac-like intestinal antibody (CIA) pattern occurs in dermatitis herpetiformis patients with normal biopsy histology<sup>5</sup>;

(3) The total IEL count may be high as a subtle feature of enteropathy in some coeliac disease patients<sup>6</sup>;

(4) Anti-reticulin and anti-endomysium antibodies are present in some healthy relatives of coeliac disease patients<sup>7</sup>;

(5) Intestinal permeability changes have also been reported in first degree relatives of coeliac disease patients.<sup>8</sup>

We recently reported that 19% of 217 non-coeliac disease patients referred for jejunal biopsy had a positive CIA pattern, and that a proportion of these (six of the nine patients who had a trial of gluten free diet) were clinically gluten sensitive, particularly when the total IEL count was high.<sup>9</sup> We have now undertaken a comprehensive evaluation of non-coeliac disease patients to establish the associations (or lack of associations) between the CIA pattern and high  $\gamma/\delta$  IEL counts, high total IEL counts, serum IgA anti-gliadin antibody, and intestinal permeability to the probe sugars lactulose and rhamnose. We have not attempted to include systematic clinical investigations of gluten sensitivity in this series.

## Methods

### SPECIMEN COLLECTION AND PROCESSING

This gastrointestinal unit provides a regional service for the clinical investigation of gastrointestinal function. In patients referred for peroral jejunal biopsy, the following protocol was used.

After an overnight fast, the patient swallowed a Watson biopsy capsule together with 15 mg of metoclopramide. When the capsule had passed to the first loop of jejunum (assessed by x ray screening), 1-2 ml intestinal fluid were drained by gravity through the tubing. The protease inhibitor, phenylmethyl sulphonyl fluoride (PMSF), 20  $\mu$ l/ml at a concentration of 0.1 M, was immediately added. The capsule was then fired to obtain a jejunal mucosal biopsy. Jejunal fluid was transferred on ice to the laboratory and

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stored at  $-70^{\circ}\text{C}$  within 10 minutes of collection. Venous blood samples were also taken. The serum was separated, aliquotted, and stored at  $-70^{\circ}\text{C}$ . A lactulose/rhamnose test of jejunal permeability was also performed.<sup>10</sup>

Jejunal mucosal biopsy specimens were orientated with the aid of a dissecting microscope and divided into two pieces. One was formalin-fixed and sent to the diagnostic pathology laboratory for subjective examination by a consultant pathologist. The other piece was embedded in OCT compound, frozen, and stored at  $-70^{\circ}\text{C}$ .

Reference values for CD3 and  $\gamma/\delta$  IEL counts were obtained from results in a subset of jejunal biopsy patients, who were eventually considered immunologically normal after investigation. Since all of these patients had been suspected of having coeliac disease, we validated this reference range by studying small bowel (duodenal) biopsy specimens from a further 26 patients, none of whom had symptoms of small bowel disease. Specimens from the distal second part of the duodenum were collected at upper gastrointestinal endoscopy, embedded in OCT compound, and frozen.

#### CLINICAL DIAGNOSIS

Some of the series were patients with coeliac disease who were having follow up biopsies to assess their response to gluten free diet. The final diagnosis for the remaining patients was made two to three months after the biopsy procedure from examination of the case records, and without knowledge of the results of immunological investigations. These non-coeliac patients were grouped as follows:

##### *Not coeliac disease*

Patients in whom a diagnosis of coeliac disease had previously been made on inadequate criteria (no biopsy or normal biopsy findings); in the final analysis they have all been classified as healthy.

##### *Family members*

First degree relatives of coeliac disease patients, subsequently shown to be normal.

##### *Inflammatory bowel disease*

Patients with Crohn's disease or ulcerative colitis.

##### *Idiopathic diarrhoea*

Patients with chronic, watery diarrhoea in whom no firm diagnosis was made after full investigation by a consultant gastroenterologist.

##### *Oral ulceration*

Patients with severe aphthous ulceration (HIV negative), referred for malabsorption investigations by dental surgeons but in whom no intestinal disease was present after full investigation.

##### *Miscellaneous*

This group comprised patients with various severe organic diseases, including small bowel bacterial colonisation, colorectal cancer, radiation enterocolitis, drug induced diarrhoea, collagenous colitis, and eczema.

##### *Nutritional deficiencies*

These subjects had anaemia or weight loss that was ultimately attributed to a poor diet.

##### *Irritable bowel syndrome*

These patients had symptoms of abdominal pain, bloating, and variable bowel habit with no identifiable organic cause.

##### *No abnormality detected*

A few patients with trivial symptoms, psychiatric disease, or constitutional short stature were placed in this group.

#### DUODENAL BIOPSY PATIENTS STUDIED

Duodenal biopsy specimens were collected from 26 patients (17 women, nine men; age range 16–87, mean 63.7 years) without small bowel disease, who had diagnostic upper gastrointestinal endoscopy. Their diagnoses were duodenal ulcer (4), gastric ulcer (4), hiatus hernia (3), oesophagitis (4), other gastrointestinal disease (3), no abnormality detected (8).

#### COUNTS OF TOTAL IEL LYMPHOCYTES

A differential count of cells within the villus epithelium was performed with a Leitz microscope and a  $\times 100$  immersion lens. At least 1000 enterocytes per biopsy specimen were counted and the results were expressed as numbers of lymphocytes per 100 villus enterocytes.<sup>6</sup> Normal values are 10–40 IEL per 100 villus enterocytes. Formal counts were performed in all 77 patients without coeliac disease and in the five specimens from treated coeliac disease patients with normal villous architecture.

#### COUNTS OF CD3 POSITIVE AND OF $\gamma/\delta$ IEL

Serial 7  $\mu\text{m}$  cryostat sections mounted on poly-L-lysine (Sigma Chemical Co, Poole, Dorset) coated slides were dried and then fixed in fresh acetone for 30 minutes at room temperature. The staining technique was as follows. After being rehydrated in Tris buffered saline (TBS, pH 7.6) and blocked with rabbit serum (SAPU, Carlisle, Scotland) in TBS, monoclonal antibodies were then applied for 60 minutes: TCR- $\delta 1$  (T Cell Sciences, Cambridge, MA) at dilution 1/80, or CD3 (SAPU) at dilution 1/20; followed by biotinylated rabbit anti-mouse IgG (DAKO, High Wycombe, UK) at dilution 1/400, for 60 minutes. The sections were treated with StrepABComplex (DAKO) for 60 minutes, and the reaction was visualised using diaminobenzidine (DAB) as a substrate. Sections were counterstained with Gill's number 1 haematoxylin. The appearances of a typical biopsy

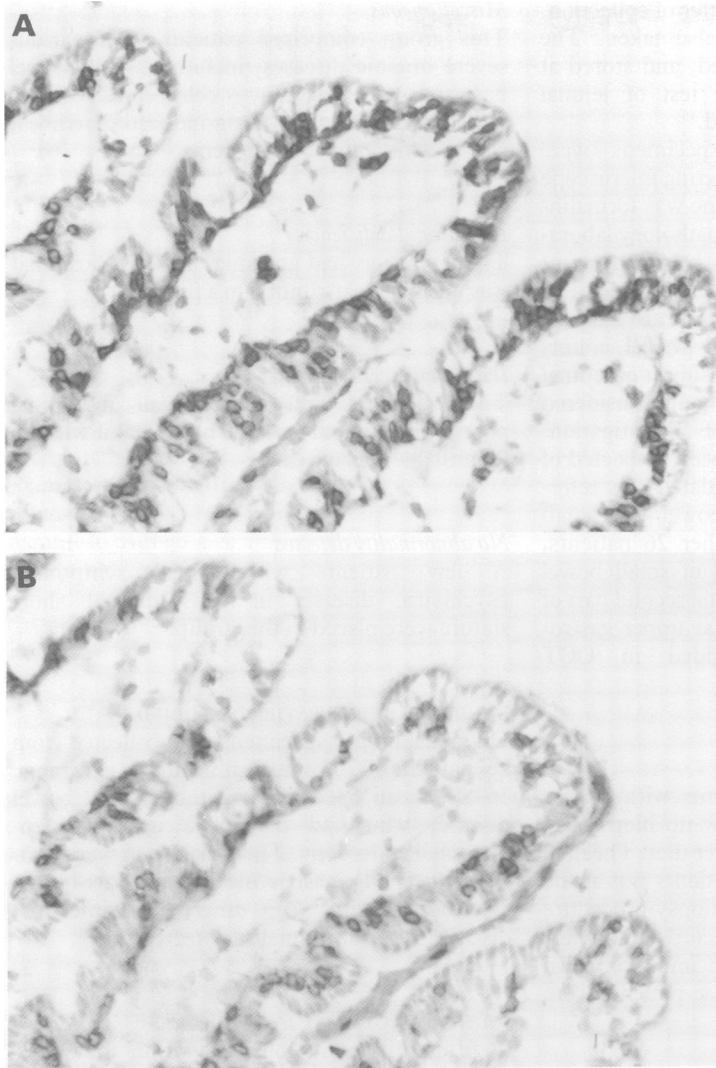


Figure: Serial cryostat sections of jejunum from a non-coeliac disease patient with a high total intraepithelial lymphocyte count. Immunoperoxidase staining of intraepithelial lymphocytes with antibodies to CD3 (Fig A) and to T cell receptor  $\delta 1$  (Fig B).

with a high count of  $\gamma/\delta$  IEL are illustrated in the Figure.

A Leitz-TAS plus computerised image analysis system (with a TASIC software operating system) was used for cell counts in frozen sections, and the results were expressed as the total cell count per mm of villous epithelium. Only those specimens in which at least 5 mm of epithelium could be counted were considered technically acceptable; with duplicate counts by separate observers, the mean difference in cell count was 17%, range 3–32%. Details of how the reference range was derived are presented below.

#### IMMUNOGLOBULIN AND ANTIBODY ASSAYS

In specimens of jejunal fluid, concentrations of IgA and IgM, and titres of IgA and IgM antibodies to gliadin (gift from Dr H Weiser), ovalbumin, and  $\beta$ -lactoglobulin (Sigma Chemical Co, Poole, Dorset, UK) were assayed by enzyme linked immunosorbent assays (ELISA) as previously described.<sup>11</sup> Levels of IgA and IgG anti-gliadin in serum were also measured by ELISA.

#### CLASSIFICATION OF ANTIBODY DATA

'Normal' values for the various antibody assays were based on results in 28 immunologically normal subjects who had been studied previously.<sup>11</sup> When jejunal fluid antibody studies showed a high value for IgM anti-gliadin, together with high titres of at least two other intestinal antibodies characteristic of coeliac disease, IgA anti-gliadin, IgM anti-ovalbumin, IgM anti- $\beta$ -lactoglobulin, the specimen was designated as CIA positive (CIA+), and all other specimens were considered CIA negative (CIA-).

#### SUGAR PERMEABILITY TEST

Concentrations of lactulose and rhamnose in urine were assayed by high pressure liquid chromatography (HPLC) and the percentage of lactulose and rhamnose excreted was expressed as the lactulose:rhamnose ratio. Normal values are  $\leq 0.040$ .

#### STATISTICAL METHODS

Differences in cell counts between groups were assessed by the two tailed Mann-Whitney U test. Comparisons of frequency tables of positivity for candidate markers was by the  $\chi^2$  test with Yates's correction.

## Results

#### JEJUNAL BIOPSY PATIENTS STUDIED

Material suitable for the full range of immunological investigations was obtained from 77 patients without coeliac disease (49 women, 28 men; age range 17–77, mean 37.3 years). Details of the patients, their diets, final diagnoses, biopsy histological classification, and sugar permeability test results are given in Table I.

TABLE I Patients studied, classified by final diagnosis and biopsy specimen histology

Diagnosis	No of patients	Age (y) mean (range)	Sex F/M	Diet		Histology			Permeability	
				Normal	Gluten free	Normal	Minor changes*	PVA	Normal	Abnormal
Not coeliac disease	4	30.6 (17–64)	2/2	2	2	3	1		4	
Family member	4	26.8 (17–44)	4/–	3	1	4			4	
Inflammatory bowel disease	6	38.5 (23–65)	3/3	6		5	1		2	4
Idiopathic diarrhoea	14	32.6 (21–70)	6/8	13	1	12	2		13	1
Oral ulceration	4	27 (19–52)	3/1	4		3	1		3	1
Miscellaneous	19	42.6 (19–77)	13/6	19		18	1		17	2
Nutritional deficiency	4	35 (30–46)	4/–	3	1	4			4	
Irritable bowel syndrome	16	38 (21–71)	10/6	16		16			15	1
No abnormality detected	6	30 (21–52)	4/2	6		6			6	
Coeliac disease†	15	42.7 (16–86)	12/3	2	13	5	2	8	7	8

\*Minor non-specific histological abnormalities.

†15 biopsies in 14 patients.

PTVA=severe partial or total villus atrophy.



As part of the technical appraisal of CD3 and  $\gamma/\delta$  cell counts, frozen sections of jejunal biopsy specimens from a group of coeliac patients (predicted to have high  $\gamma/\delta$  IEL counts) were also examined. Fifteen jejunal biopsy specimens were collected from 14 coeliac disease patients (11 women, three men; age range 16–86, mean 42.7 years). Thirteen patients had already been diagnosed and were attending for follow up biopsies (one of these had two biopsies, one while on a gluten free diet and a second during a gluten challenge); only one was a newly presenting patient.

#### REFERENCE VALUES FOR $\gamma/\delta$ IEL, AND EXPRESSION OF RESULTS (TABLE II)

A subset of 34 patients who had undergone jejunal biopsy but were considered at final assessment to be immunologically entirely normal, was used to establish interim reference ranges for jejunal CD3 IEL cells and  $\gamma/\delta$  IEL counts (per mm epithelium). Sixteen of this group had irritable bowel syndrome, 14 had trivial or transient disorders, and in four no abnormality was detected. The reference values for the upper limits of normal (mean +2 SD) thus obtained were 67.5 CD3 IEL per mm epithelium and 5.5  $\gamma/\delta$  IEL per mm epithelium.

We had to decide whether to express the results for  $\gamma/\delta$  IEL as a percentage of the total number of CD3+ IEL, as has been the practice of some other groups. However, this produced spuriously high, abnormal values in a few cases with very low total CD3 counts (presumable with deficiency of  $\alpha/\beta$  IEL rather than excess of  $\gamma/\delta$  cells). We have therefore presented data for  $\gamma/\delta$  IEL only as the unmodified count per unit length

of villus or surface epithelium, taking values >5.5 cells/mm epithelium as abnormally high.

Fourteen of the biopsy specimens from patients with coeliac disease had abnormally high  $\gamma/\delta$  IEL counts. The remaining patient (normal mucosa, on a gluten free diet, with no unusual clinical features) had a normal count; his data is presented separately from the other cases.

As shown in Table II, there were significant differences between the reference values obtained for CD3 cells in duodenal and jejunal specimens, with values for duodenum (mean 19.4 CD3+ IEL per mm epithelium, SD 11.4) significantly lower ( $p < 0.01$ ) than those for jejunum. This may reflect a true difference between these tissues, but another explanation is that those patients undergoing duodenal biopsy were significantly older than the reference jejunal biopsy group (we have previously reported low IEL counts as a feature of human immunosenescence<sup>12</sup>). However,  $\gamma/\delta$  cell counts (per mm) were similar in the two sites.

There was one endoscopy patient (a woman with an uncomplicated gastric ulcer) who had a strikingly high  $\gamma/\delta$  count at 24 per mm; in other respects her duodenal biopsy histology was entirely normal.

#### COMPARISON OF CD3 AND TOTAL IEL COUNTS

In much of the published work on latent coeliac disease, and on the spectrum of pathological expression of gluten sensitivity, IEL counts have been performed in haematoxylin and eosin stained sections, using a conventional light microscope, with results expressed as IEL per 100 enterocytes. In the future, if frozen sections and image analysis are necessary to count  $\gamma/\delta$  IEL, it may be preferable to count CD3+ cells per mm as an alternative to total IEL per 100 enterocytes. The material examined in the present series allows a direct comparison of these two techniques. As shown in Figure 2, there was a highly significant correlation between IEL counts in paraffin sections and CD3+ cell counts in frozen sections ( $r = 0.765$ ,  $p < 0.001$ ). All cases with high CD3+ cell counts had high IELs, but there were seven cases with high IEL count and normal CD3 cell count, suggesting that a non-T subset of lymphocytes may be present in the epithelium of these patients.

TABLE II Reference values for cell counts in villous epithelium (mean (SD) (range))

	CD3+ cells per mm	T cell receptor $\gamma/\delta$ cells per mm
Control group		
Jejunal biopsy patients (n=77)	33.7 (16.9) (6–68.7)	1.5 (2) (0–7.4)
Duodenal biopsy patients (n=26)	19.4 (11.4) (2.3–45.9)	1.5 (4.7) (0–24.4)
Excluding the atypical case	18.4 (10.3) (2.3–44.1)	0.6 (0.6) (0–2.5)
Coeliac disease patients (n=15)	69.2 (32.9) (31.9–120)	21.5 (16.7) (5.5–66)
Atypical case	42.2	0.2

\* $p < 0.001$ .

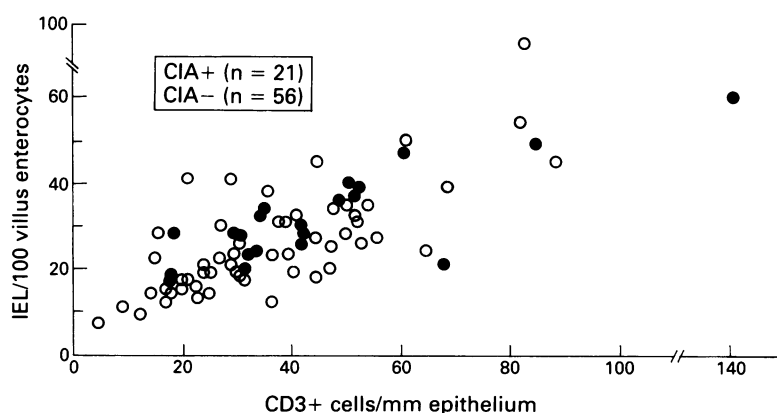


Figure 2: Intraepithelial lymphocyte (IEL) counts in 77 pairs of jejunal biopsy specimens. Total IEL counts in paraffin sections are expressed as IEL per 100 villus enterocytes; CD3+ cell counts in frozen sections are expressed as cells per mm length of villus epithelium. ● = coeliac-like intestinal antibody positive (CIA+); ○ = CIA-.

#### POSITIVE RESULTS FOR CANDIDATE MARKERS OF LATENT COELIAC DISEASE IN 77 PATIENTS WITHOUT COELIAC DISEASE

These results are summarised in Table III, with patients subdivided into diagnostic groups.

The CIA pattern of jejunal fluid antibodies was present in 21 patients. Nine patients had high counts of  $\gamma/\delta$  IEL in the villus epithelium, and conventional IEL counts were high in 13 cases. High titres of serum IgA anti-gliadin antibodies were present in 12 patients, with no obvious relationship with diagnostic groups or the other markers. The sugar permeability test was abnormal in nine cases, mainly those with mild abnormalities of jejunal pathology or with inflammatory bowel disease.

Associations between the CIA pattern, high

$\gamma/\delta$  counts and high total IEL counts, are illustrated diagrammatically in Figure 3. High  $\gamma/\delta$  IEL counts were found in six of the 21 CIA positive patients, but in only two of 56 who were CIA negative ( $p < 0.001$ ); there were no significant associations in any other marker combinations. A high total IEL count was more frequent in CIA positive patients (five of 21), than in patients negative for the CIA pattern (eight of 56), but the difference was not significant.

Thirty one patients had one or more positive result, but in 19 only a single test was abnormal. Of particular interest are patients with three positive parameters – the CIA pattern,  $\gamma/\delta$  IEL count, and total IEL count. Two of the three have been shown to be gluten sensitive.<sup>9</sup> A woman with chronic high volume diarrhoea had complete resolution of diarrhoea within two weeks of starting a gluten free diet, and her IEL count was normal in a biopsy specimen taken two months later. Another woman with recurrent severe aphthous ulceration had a trial of gluten free diet; she noticed some improvement but found the diet inconvenient and kept to it for only five weeks. Several months later, when studies of  $\gamma/\delta$  IEL in a stored biopsy specimen were performed and counts were found to be high, she agreed to take 10 g additional gluten daily for a month and had another jejunal biopsy. Pathological examination showed subtotal villus atrophy.

## Discussion

The existence of latent coeliac disease suggests that the finding of a normal jejunal biopsy does not completely exclude gluten sensitivity. As discussed above, there is some evidence that certain minor immunological abnormalities (which persist in treated coeliac disease and dermatitis herpetiformis patients after healing of enteropathy) may identify patients with latent coeliac disease. One of these changes, the CIA pattern – polyclonal upregulation of mucosal IgM responses – occurs in 20–30% of patients referred for diagnostic jejunal biopsy. We have shown that the symptoms of some CIA+ patients respond to treatment with a gluten free diet.<sup>9</sup>

In this study we have combined assessment of the CIA pattern with studies of another candidate marker of latent coeliac disease, a high count of  $\gamma/\delta$  IEL, together with several other immunological and functional tests. It is important to emphasise that there is a conceptual difference between the finding of a high total IEL count and the other aspects studied. It is now generally accepted that a high density of villus IEL (for example, as assessed by differential counts in the present study) may be the only pathological expression of gluten sensitivity in coeliac disease patients,<sup>13</sup> allowing the diagnosis of a mild but significant enteropathy. However, the pathophysiological, diagnostic, and clinical importance of positive findings for the other parameters examined are still uncertain.

We have found a statistically significant association between a positive CIA pattern and a high density of  $\gamma/\delta$  IELs. The CIA pattern was also correlated (but not significantly) with a high total IEL count. We have also confirmed our previous findings that patients with subtle immune changes in gut humoral immunity have entirely normal titres of serum IgA anti-gliadin antibody.<sup>9,11</sup> A positive sugar permeability test is independent of the other features; it is mainly abnormal in patients with inflammatory bowel disease and alcohol or NSAID related problems.

Around 40% of patients referred for diagnostic jejunal biopsy in whom routine biopsy pathology was normal, had positive results for one or more

TABLE III Diagnostic groups: numbers of cases positive for candidate markers of latent coeliac disease

Final diagnosis	No	CIA+ cases	High $\gamma/\delta$ counts	High IEL counts	Abnormal L/R test	High serum AGA titres
Not coeliac disease	4	2	1	1		
Family member	4	1				1
Inflammatory bowel disease	6	2	1		4	2
Idiopathic diarrhoea	14	4	3	4	1	2
Oral ulceration	4	2	1	2	1	1
Miscellaneous	19	4		2	2	3
Nutritional deficiency	4	2	1	1		
Irritable bowel syndrome	16	3	2		1	2
No abnormality detected	6	1		3		1
Coeliac disease						
Normal diet	1	1	1	ND	1	1
Gluten challenge	1	1	1	ND	1	1
Gluten free diet	13	8	12	ND	6	4

CIA=coeliac-like intestinal antibody; IEL=intraepithelial lymphocytes; L=lactulose; R=rhamnose; AGA=anti-gliadin antibody.

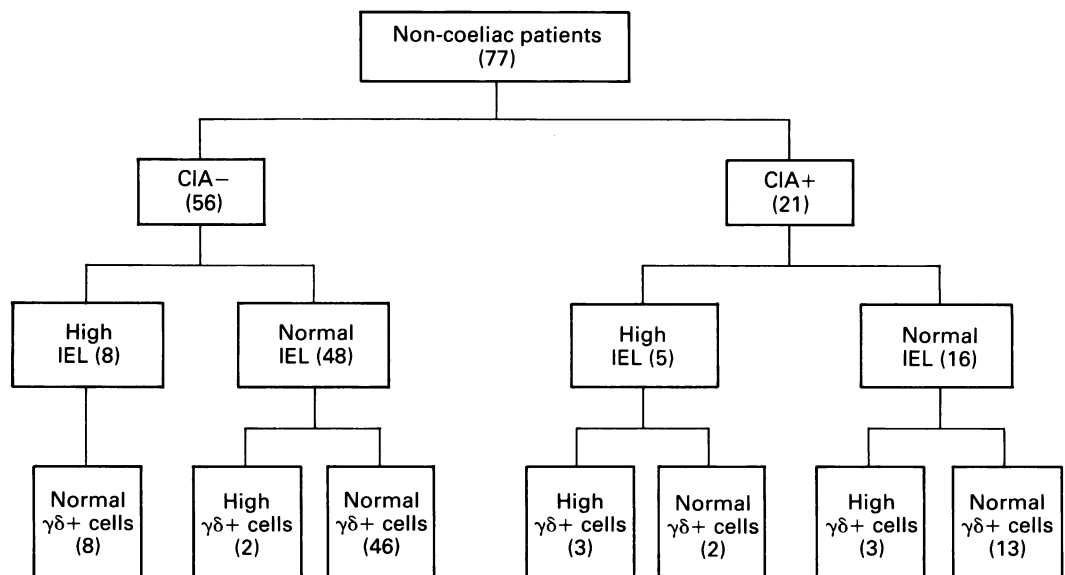


Figure 3: Associations between the coeliac-like intestinal antibody (CIA) pattern – high  $\gamma/\delta$  counts, and high total intraepithelial lymphocyte (IEL) counts – in 77 non-coeliac patients.

of the tests that have been proposed as indices of latent coeliac disease. Although we have obtained evidence of clinical and mucosal gluten sensitivity in a few of these patients,<sup>9</sup> this has been on an opportunistic basis, unsystematic and uncontrolled. Induction of severe enteropathy by extra dietary gluten would be unequivocal proof of gluten sensitivity, but this raised ethical issues and may be clinically unacceptable to symptomatic patients. Acute enteral or rectal gluten challenge, monitored by multiple biopsies, should be more practicable. However, since the histopathological effects of dietary gluten in clinically gluten sensitive/normal jejunal biopsy patients are strikingly different from those in classic coeliac disease, it is entirely possible that the pathological changes produced by acute gluten challenge in the jejunum or rectum will be equally different in latent/potential coeliac disease subjects and those with classic coeliac disease.

With the currently available range of tests, studies of a single putative marker cannot be expected to identify all cases of latent coeliac disease. The two best indices are likely to be a positive CIA pattern and high  $\gamma/\delta$  count. However, these are not present even in all classic coeliac disease patients. We found one of 14 untreated patients with coeliac disease and one of six dermatitis herpetiformis patients were CIA negative,<sup>9</sup> and one otherwise completely typical man with coeliac disease in the present series (who also has insulin dependent diabetes) had no  $\gamma/\delta$  IEL. Although logistically difficult, the identification of potential coeliac disease patients still requires studies of several candidate markers, using jejunal fluid for ELISA studies and frozen sections of a mucosal biopsy specimen for T cell receptor staining.

A high total IEL count in a haematoxylin and eosin stained section may be the result of expansion of one or more of the three main subsets of lymphocytes within the villus epithelium of the small intestine: (i) CD3 positive (T cells) IEL which utilise the  $\alpha/\beta$  T cell receptor – numbers of these rise and fall in coeliac patients with gluten ingestion and exclusion; (ii) CD3 positive (T cells) IEL which utilise the  $\gamma/\delta$  T cell receptor – generally, counts of these cells are high in coeliac patients, irrespective of diet; (iii) a small proportion of IEL are CD3 negative cells, with no T cell receptors – their nature, in man, is uncertain. In a few of these patients, the total IEL count was high with the CD3 cell count normal, suggesting that expansion of the atypical, non-T IEL subset may occasionally occur; further patients will need to be studied to establish the clinical importance of this finding.

The CIA pattern and a high  $\gamma/\delta$  IEL count occur independently in some non-coeliacs, but also coexist more frequently than expected by chance. These phenomena may be due to separate, intrinsic, genetically determined aberrations of the constituent lymphocyte populations of the mucosal immune system, which happen to occur together in most coeliac disease patients. Alternatively, the possession of both aberrations may increase the likelihood of full expression of enteropathy in an individual who is genetically predisposed by virtue of an indepen-

dent gene for gluten sensitivity. These aberrations may be constitutively expressed, or may be detectable only in situations of mucosal immune activation. Whatever the underlying mechanism, it is clear that the repertoire selected from the full range of potential immune cells and molecules includes intestinal IgM antibodies and  $\gamma/\delta$  IEL in coeliac disease patients, whereas these are not utilised in most of those without coeliac disease.

We have discussed elsewhere<sup>9</sup> the range of possible mechanisms of the CIA pattern, including a primary defect of maturation or IgA-IgM switch of B cells and immunoregulatory T cell dysfunction. An expanded  $\gamma/\delta$  IEL population may be genetically determined.<sup>3,4</sup> High counts of  $\gamma/\delta$  IEL, but not of  $\alpha/\beta$  IEL, were found in healthy relatives of coeliac disease patients who possessed the extended haplotype of coeliac disease.<sup>14</sup> Alternatively,  $\gamma/\delta$  IEL may represent an expanded population controlled by local regulatory factors, perhaps triggered by gluten.<sup>15,16</sup> However,  $\gamma/\delta$  IEL have very limited diversity (and thus limited capacity for antigen recognition)<sup>17</sup> and they are not gluten specific.<sup>18</sup> The fact that a high density of  $\gamma/\delta$  IELs was found in a patient with latent coeliac disease years before the development of enteropathy,<sup>3</sup> and our finding of high  $\gamma/\delta$  IEL counts in several patients without coeliac disease, argue against their involvement in mucosal damage.

Further elucidation of the relationships between the immunological phenomena described and the clinical entity of coeliac disease will require not only direct, *in vivo* investigations of gluten sensitivity in potential coeliac disease patients, but also characterisation of the genetic make-up of both coeliac and non-coeliac patients, who do or do not have the various immune abnormalities described above.

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