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T cell receptor repertoire in the peripheral blood and intestinal mucosa of coeliac patients

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SUMMARY

The $\alpha\beta$ and $\gamma\delta$ T cell receptor (TCR) repertoire in the peripheral blood and intestinal mucosa of six coeliac children and six age-matched controls was analysed by reverse transcription and polymerase chain reaction (PCR). No TCR $\alpha\beta$ and $\gamma\delta$ restriction was observed in coeliacs and controls. However, Vγ3 was expressed only in coeliac peripheral and intestinal T cells. Vδ2 was strongly expressed in coeliacs and scarcely transcribed in control cells. The unique expression of these $\gamma\delta$ TCR in coeliac patients suggests that Vγ3 and perhaps Vδ2 TCR-bearing lymphocytes may play a role in the pathogenesis of coeliac disease.

Keywords coeliac T cell receptor $\gamma\delta$ T cells autoimmunity restricted usage

INTRODUCTION

The role of autoimmune mechanisms in the development of coeliac disease is controversial. Several lines of evidence support autoimmune etiology: polygenic inheritance [1], HLA linkage [2], specific autoantibodies [3–5], association with other autoimmune disorders [6,7], similarity between extra-intestinal manifestations and common clinical signs in autoimmune diseases [8] and T lymphocyte infiltration and activation within the small bowel mucosa [9]. However, all these arguments are indirect, and direct proof for the equation of coeliac sprue with autoimmunity is still lacking. Moreover, autoimmune organ-directed diseases are progressive and end-organ destruction is complete and irreversible. In coeliac disease, removal of a known, external dietary antigen results in complete healing of the mucosa. Recently, the possibility that one or a few T cell clones may be involved in initiating autoimmunity has been raised. The restricted usage of T cell receptor (TCR) V genes by autoreactive T cells was demonstrated in experimental allergic encephalomyelitis (EAE) [10,11], murine collagen-induced arthritis (CIA) [12], autoimmune thyroid disease [13,14] and multiple sclerosis (MS) [15]. Such observations have resulted in the hypothesis that specific combinations of TCR sequences are detrimental to the induction of autoimmune diseases, and have led to the development of immunomodulatory treatments directed against diseases associated with the expression of specific TCR [16,17]. In an attempt to look for additional indirect evidence for autoimmune features in coeliac disease, we investigated the clonality of T cells which had been infiltrated into coeliac intestine. We used polymerase chain reaction (PCR) to analyse TCR structural composition.

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PATIENTS AND METHODS

Patients

Peripheral blood and intestinal specimens were obtained from patients followed by the Paediatric Gastroenterology and Nutrition Unit (Carmel Medical Centre, Haifa, Israel). Six children (mean age 3 years, range 1–6 years; male : female 1 : 1) with coeliac disease according to the ESPAGAN criteria [18] were evaluated. Three children were on a normal, gluten-containing diet whereas the other three had been on a gluten-free diet for at least 1 year. All blood samples were obtained on the day of the intestinal biopsy. Six children (mean age 3·5 years, range 2–7 years; male:female 1 : 1), with non-specific abdominal pain served as controls. Informed consent was obtained from parents and the study was approved by the Ethical Committee, Technion-Israel Institute of Technology.

Small intestinal biopsy

The intestinal biopsies were performed with a GIF-X10 endoscope (Olympus Corp., Tokyo, Japan). Histopathological slides (haematoxylin and eosin staining) were examined by a single pathologist blinded to the clinical and laboratory findings. All three coeliac children, on normal diet, had grade III–IV small intestinal atrophy [19]. The other three coeliacs, on

gluten-free diet, and the six controls had normal intestinal biopsy. An additional duodenal sample was snap-frozen for further processing.

Peripheral blood mononuclear cells

Peripheral blood mononuclear cells (PBMC; 15 ml) were separated from heparinized blood by density gradient centrifugation using Ficoll–Hypaque (Pharmacia, Uppsala, Sweden) and stored in liquid nitrogen until RNA extraction.

PCR analysis of TCR genes

The methodology has been previously described [13,14]. Briefly, total cellular RNA of PBMC and intestinal biopsies was extracted with guanidinium thiocyanate and phenol (RNAzol B; Cinna Bioteck Laboratories Int., Friends Wood, TX). cDNA transcripts were prepared with oligodeoxythymidine priming and avian reverse transcriptase in the presence of RNAse inhibitor (40 U/20 ml; Promega, Madison, WI). Eighteen Vα, 19 Vβ, six Vγ and five Vδ gene family-specific 5′ oligonucleotide primers were synthesized using an Applied Biosystems (Foster City, CA) DNA synthesizer, and paired with 3′ primers matched to Cα, Cβ, Cγ or Cδ genes, respectively. The Vα, Vβ, Vγ and Vδ gene family-specific primers and probes were previously reported [14,20].

The denatured cDNA was amplified with Taq DNA polymerase, Taq polymerase buffer, and 1·5 mM/*l* of each nucleotide in 35 amplification cycles [13,14]. Negative controls included tubes without cDNA or irrelevant cDNA. The amplified products were electrophoresed on 1·5% agarose gels with ethidium bromide and visualized under UV light. To increase the sensitivity and specificity of the assay, the agarose gels were blotted onto nitrocellulose membranes, baked, and then hybridized with ^{32}P-ATP-labelled oligonucleotide probes (5×10^5 ct/min of probe/ml) specific to the Cα, Cβ, Cγ or Cδ regions, respectively, and internal to the TCR products, as described elsewhere [13,14]. After washing, the blots were exposed to x-ray film with an intensifying screen for 3–24 h.

RESULTS

TCR Vα and Vβ amplification

The Vα gene family utilization has been previously found to be 17·3 ± 0·3 of 18 gene families examined [13]. In this study PBMC derived from controls and from both active and non-active coeliac patients expressed comparable results: 17·1 ± 0·4 gene families were transcribed (Fig. 1). In 10 out of the 12 children examined, 16 positive Vα gene families were observed (Vα 1–16), while only two Vα17 and Vα18 were found. Two samples were negative for Vα9 and other two negative for Vα4. The present study revealed 15·4 ± 3·4 active Vβ genes in the peripheral blood of active and inactive coeliacs and in controls (Fig. 2), which is not significantly different from previous published results [13]. Vβ19 was not transcribed in 10 out of the 12 patients studied, while Vβ10 was negative in six. All other Vβ gene families were expressed in the three groups. In intestinal biopsies obtained from coeliac patients with active and non-active disease, and from control intestinal biopsies 14·2 ± 2·8 Vα gene families and 15·1 ± 2·5 Vβ gene families were transcribed (Figs 3 and 4). No statistically significant differences in TCR V$\alpha\beta$ gene utilization were discerned between coeliacs and controls or between peripheral or intestinal T cells. However, qualitative differences were found between Vα and Vβ gene expression in peripheral blood and intestinal biopsies: Vα1, Vα9 and Vα17,18 were not transcribed in intestinal T cells, while they were expressed in the periphery. Vα4 was not transcribed in nine of the 12 biopsies, Vα7 and Vα10 in five, and Vα16 in three. Vβ2 and Vβ3 were scarcely

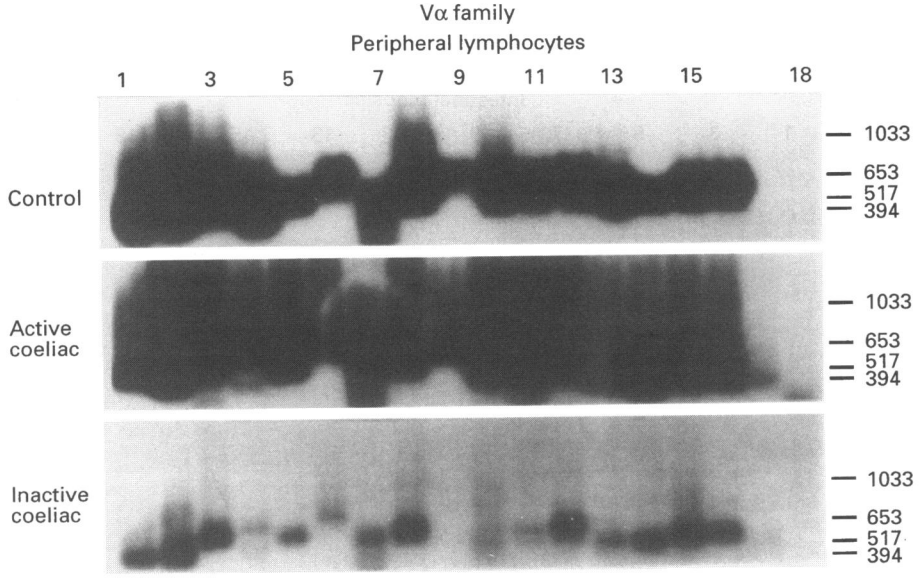

Fig. 1. Vα T cell receptor amplification in the peripheral blood mononuclear cells (PBMC) derived from a representative normal control, and representative active and inactive coeliac patients. Each lane represents hybridization with a family-specific Vα probe, following amplification with a family-specific Vα 5′ primer and a 3′ Cα primer. The utilization of 18 different Vα T cell receptor gene families is presented. The nomenclature used for the α primers is described in [14]. Vα1 is represented in lane 1, Vα2 in lane 2, Vα3 in lane 3, etc.

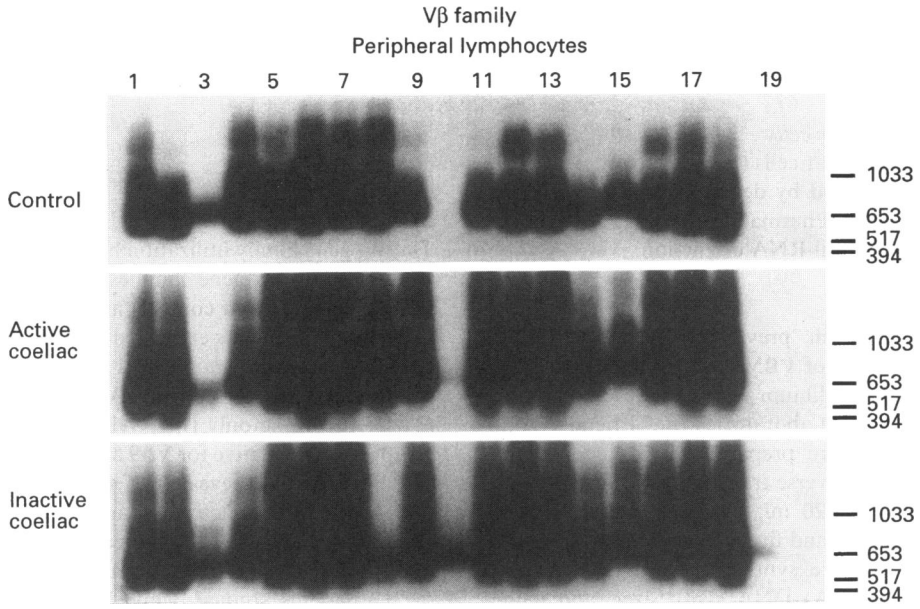

Fig. 2. Vβ T cell receptor amplification in peripheral blood mononuclear cells (PBMC) of normal controls, active and inactive coeliac patients. A representative case from each group is demonstrated. Each lane represents hybridization with a family-specific Vβ probe, following amplification with a family-specific Vβ 5′ primer and a 3′ Cβ primer. The utilization of 19 different Vβ T cell receptor gene families is presented. The nomenclature used for the β primers is described in [14]. Vβ1 is represented in lane 1, Vβ2 in lane 2, Vβ3 in lane 3, etc.

utilized by intestinal T cells but strongly expressed in peripheral blood. Vβ10 and Vβ19, which were used by some but not all peripheral T cells, were not used at all in the biopsies.

TCR Vγ and Vδ transcription in peripheral blood and intestinal biopsies

Out of six Vγ genes examined, three (Vγ1.2, 1.2-1.4 and 2) were transcribed in control and coeliac PBMC and in the corresponding intestinal lymphocyes, while an additional gene, Vγ3, was transcribed only by peripheral and intestinal T cells derived from coeliac patients (Fig. 5). All coeliac samples were positive for Vγ3, but none of the control samples. Out of five Vδ gene families examined, two (Vδ1 and Vδ3) were transcribed in both control and coeliac mucosal and peripheral lymphocytes, while a third gene family (Vδ2) was strongly expressed in all coeliac samples, scarcely utilized in two out of six control biopsies, and was not detected in the peripheral blood of the six controls (Fig. 6).

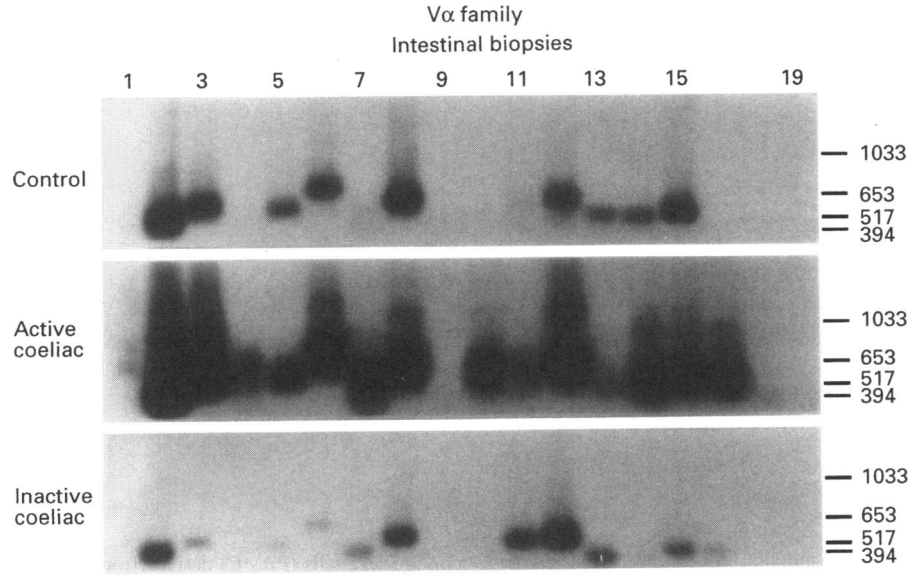

Fig. 3. Vα T cell receptor amplification in intestinal lymphocytes derived from representative control, active and inactive coeliac patients. Each lane represents hybridization with a family-specific Vα probe, following amplification with a family-specific Vα 5′ primer and a 3′ Cα primer. The utilization of 18 different Vα T cell receptor gene families is presented.

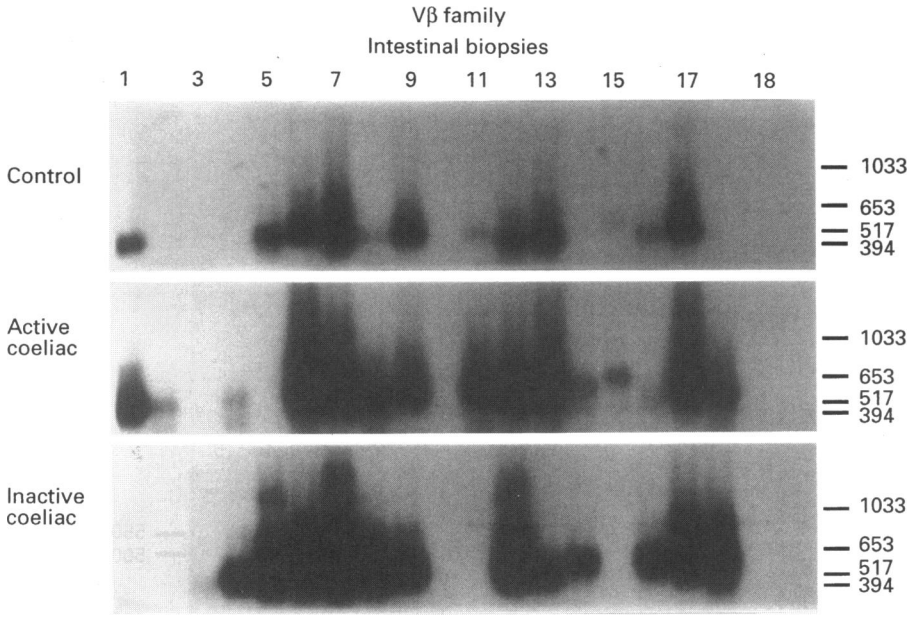

Fig. 4. Vβ T cell receptor amplification in intestinal lymphocytes derived from representative control, active and inactive coeliac patients. Each lane represents hybridization with a family-specific Vβ probe, following amplification with a family-specific Vβ 5′ primer and a 3′ Cβ primer. The utilization of 19 different Vβ T cell receptor gene families is presented.

DISCUSSION

Coeliac disease is an excellent model of nature for unraveling immunopathologic events in the gut. After gluten challenge, there is a striking migration of T cells into the epithelial and the lamina propria compartments with a differential distribution of αβ or γδ TCR-bearing lymphocytes between the two compartments. Our study was specifically designed to examine qualitative changes of the T lymphocytes taking place in the coeliac intestinal mucosa as a whole. The observed Vγ3 and Vδ2 TCR-expressing lymphocytes could originate from any of the two intestinal compartments.

The differential importance of the αβ *versus* γδ bearing

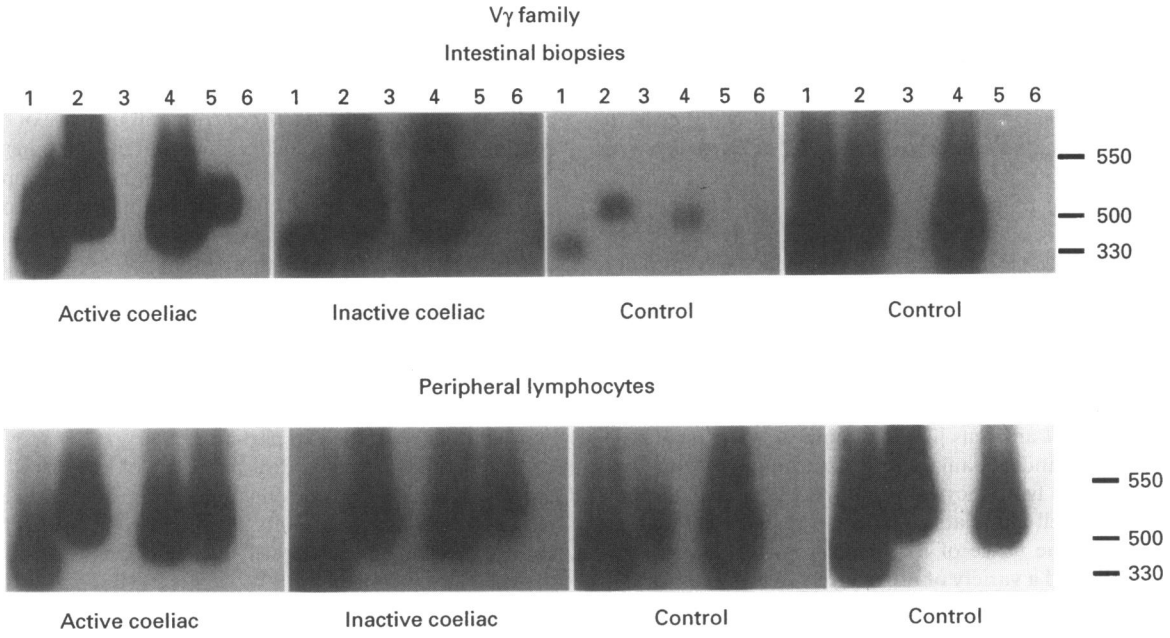

Fig. 5. Vγ T cell receptor amplification in intestinal and peripheral lymphocytes derived from two representative controls, one representative active and one representative inactive coeliac patient. Each lane represents hybridization with a family-specific Vγ probe, following amplification with a family-specific Vγ 5′ primer and a 3′ Cγ primer. The utilization of six different Vγ T cell receptor gene families is presented. The nomenclature used for the γ primers is described in [20]. Vγ1.2 is represented is lane 1, Vγ1.2(1.4) is represented in lane 2, Vγ1.8 is represented in lane 3, Vγ2 is represented in lane 4, Vγ3 in lane 5 and Vγ4 in lane 6.

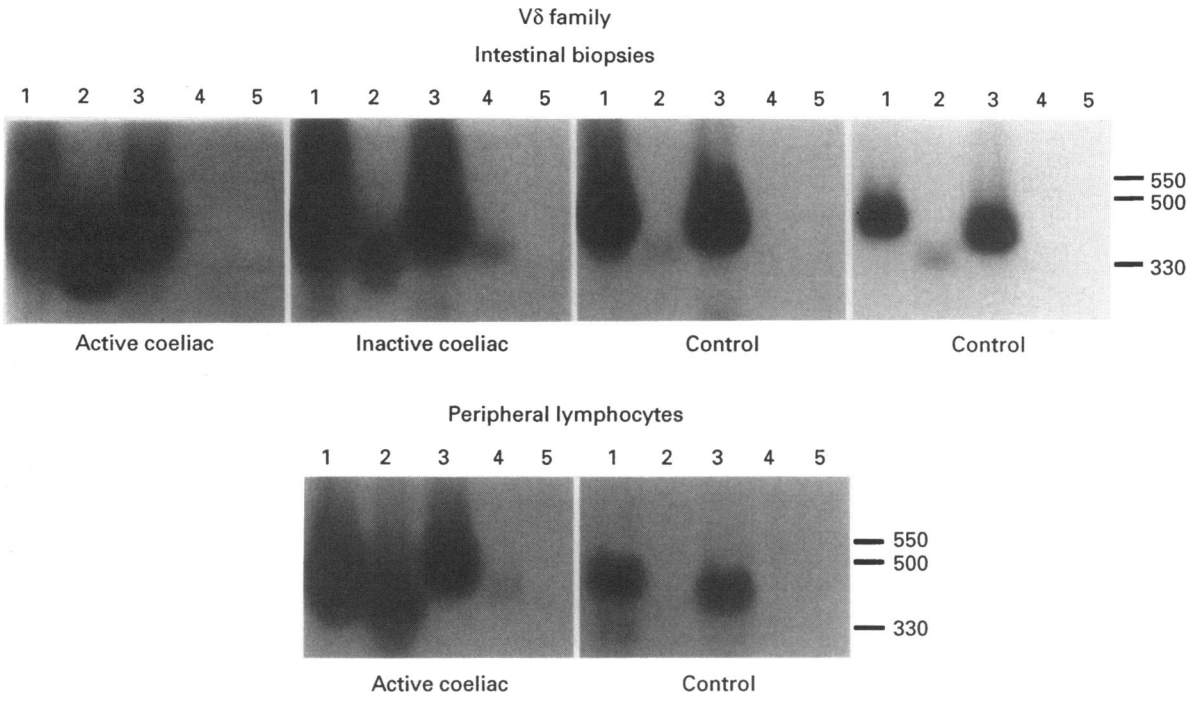

Fig. 6. Vδ T cell receptor amplification in intestinal and peripheral lymphocytes derived from two representative controls, one representative active coeliac patient and one representative inactive coeliac patient. Each lane represents hybridization with a family-specific Vδ probe, following amplification with a family-specific Vδ 5′ primer and a 3′ Cδ primer. The utilization of five different Vδ T cell receptor gene families is presented. The nomenclature used for the δ primers is described in [20]. Vδ1 is represented in lane 1, Vδ2 in lane 2, Vδ3 in lane 3, etc.

intestinal lymphocytes in coeliac disease is controversial: Kutlu *et al.* [21] have found that the number of TCR $\alpha\beta$ but not $\gamma\delta$ lymphocytes correlate with the grade of villous atrophy in active coeliac patients, and therefore suggested that these cells are directly involved in coeliac pathogenesis. Other investigators, however, have shown the importance of $\gamma\delta$ T cells in coeliac disease either as causative agents [22] or as involved in its prevention [23].

The cytopathic potential of $\gamma\delta$ lymphocytes, their ability to respond to stress proteins of damaged cells [24], their association with genetic markers for coeliac susceptibility [25] and their ability to abrogate oral tolerance [26] suggest that they may be actively involved in the induction of enteropathy. However, their increment in normal mucosa from latent coeliac patients, their persistence throughout treatment in the absence of gluten, and their inability to react to putative autoimmune target antigens [23] may point to their participation in protection and/or damage repair. Although the function of intestinal $\gamma\delta$ T lymphocytes is not yet elucidated, several subsets of this cell population have been demonstrated in coeliac disease. The usage of Vδ1 gene segment [27], Vγ3 δ3 expressing cells and a variety of other Vγ Vδ bearing clones [28] has been immunocytochemically observed. In addition, the cytotoxic effects of these cells on different targets have been demonstrated [29]. Recently T cells expressing the Vδ2 gene were demonstrated to respond to the 65-kD heat shock protein in lesions of patients with MS and rheumatoid arthritis (RA) [24]. Similar mechanisms may be involved in the coeliac mucosa.

Our study demonstrates lymphocytes transcribing Vγ3 and to some extent Vδ2 TCR, in coeliac patients and not in controls. Their existence is independent of disease activity, and they are similarly distributed in both the systemic and intestinal compartments.

The recently reported [30] concomitant increase of $\gamma\delta$ TCR-bearing lymphocytes in coeliac mucosal and peripheral compartments emphasizes their interdependency, and supports the absence of their site specificity as found in the present study. The coeliac repertoire of $\gamma\delta$ TCR expression differs from that observed in organ-specific autoimmune diseases such as EAE, thyroid autoimmune diseases, RA and MS. In these diseases specific T cells are confined to 'active' lesions, are almost exclusively utilized, and therefore are believed to be causative of the autoimmune disease. In contrast, in coeliac patients the restricted T lymphocytes are found also in the systemic circulation, and the restriction in partial.

The significance of the distribution of the T cell subsets expressing unique Vγ3 and enhanced Vδ2 in coeliac disease is as yet unknown. Their clonality and their role, whether pathogenic, defensive or neutral, will await further studies.

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