

Increase in T-Cell Subsets of Oral Mucosa: a Late Immune Response in Patients with Treated Coeliac Disease?*

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Background and aims: In coeliac disease, the gut involvement is gluten-dependent. Following the introduction of a gluten-free diet, inflammatory cell infiltration decreases in the small intestinal mucosa. Our hypothesis was that the oral mucosa might mirror the changes found in coeliac disease similarly to the mucosa of the small intestine. Thus, the number of inflammatory cells in the oral mucosa would decrease in patients with coeliac disease on a gluten-free diet. **Methods:** The distribution CD45RO⁺ and CD3⁺ T cells, T-cell subpopulations (CD4⁺, CD8⁺, T-cell receptor (TCR)αβ⁺ and TCRγδ⁺ cells) and HLA DR expression were studied in the buccal mucosa of 15 untreated and 44 gluten-free diet treated coeliac disease patients, and of 19 controls. All 15 patients with untreated coeliac disease were immunoglobulin (Ig)A endomysial antibody positive and all 44 patients on gluten-free diet except one were endomysial antibody negative, as were all control subjects. **Results:** Untreated coeliac disease patients did not differ from controls in the densities of CD45RO⁺ cells, CD3⁺ cells or of T-cell subsets. In contrast, in treated coeliac disease patients, a significant increase in the numbers of mast cells, CD3⁺ and CD4⁺ lymphocytes was found in the lamina propria of oral mucosa as compared with patients with untreated coeliac disease and controls. The increase in CD3⁺ T cells was in part owing to an increase in lymphocytes expressing no TCR. No differences were found in the expression of human leucocyte antigen (HLA) DR in the epithelium or in the lamina propria in the patient groups studied or in the controls. In treated coeliac disease patients only a few TCRγδ⁺ T cells were found intraepithelially and in the lamina propria, but these cells were not detected in the lamina propria of oral mucosa of patients with untreated coeliac disease or in the controls. **Conclusions:** The infiltration of T cells into oral mucosa was increased in treated coeliac disease patients in spite of adherence to a gluten-free diet. Because the CD3⁺ T cell count was higher than those of the TCRαβ⁺ and TCRγδ⁺ T cells, there must be other cells involved, probably natural killer (NK) cells. The increase in T-cell subsets in the treated coeliac disease patients seems not to result from poor dietary compliance, but might occur as a late immune response in coeliac disease and reflect chronic immunologic stimulation followed by regeneration of memory T cells.

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INTRODUCTION

Coeliac disease, or gluten-sensitive enteropathy, is histologically expressed in the gut by a wide variety of T-cell-mediated pathological features, ranging from low-grade enteropathy to flat jejunal mucosa. It is provoked in genetically susceptible individuals by dietary exposure to wheat gluten and similar proteins in other closely related cereals [1–3]. A gluten-free diet results in clinical as well as histological recovery. Although the major site of gut involvement in coeliac disease is the jejunal mucosa, changes have also been observed in the gastric and rectal mucosa [1]. Oesophageal impairment has also been shown to be related to the ingestion of gluten in patients with coeliac disease [4].

In coeliac disease, it has been suggested that activated T cells in the epithelium and lamina propria of the gut, mostly bearing the TCR $\alpha\beta$, may play a central role in the development of villous atrophy [5]. Gluten-specific mucosal CD4⁺ T cells expressing TcR $\alpha\beta$ are activated in the lamina propria of the small intestine but proliferate in the epithelium, as do the TCR $\gamma\delta$ T cells [6]. During a gluten-free diet, the intraepithelial lymphocytes in the gut mucosa decrease, but the overrepresentation of TCR $\gamma\delta$ intraepithelial lymphocytes in the epithelium remains [6].

Oral recurrent aphthous ulcerations, as well as nonaphthous ulcerations, have been described in patients with coeliac disease [7], and oral mucosal lesions have also been detected in treated coeliac disease patients [8]. In a recent study on dermatitis herpetiformis, the authors demonstrated that intraepithelial

TCR $\gamma\delta$ T lymphocytes of oral mucosa were rather rare in untreated and gluten-free diet treated patients and in controls [9].

Now, we were interested in finding the pattern of T-cell subsets in oral mucosa and whether this shows changes typical only to coeliac disease. We hypothesized that oral mucosa, although consisting of a stratified squamous, and not of a single epithelial cell layer, mirrors coeliac disease development and improvement during gluten-free diet treatment in the same way as has been shown to occur in the small intestinal mucosa [3]. As far as the authors know, this is the first report regarding the T-cell subsets of oral mucosa in coeliac disease.

MATERIALS AND METHODS

Patients. The coeliac disease patients with gluten-free diet treatment (44 patients; mean age 46.7 years; range 24–65 years; female : male ratio 36 : 8) were participants in a large earlier study on oral mucosa and coeliac disease [8]. The duration of coeliac disease was less than 1 year for 1/44 patients (2.3%), between 1 and 2 years for 8/44 (18.2%), less than 5 years for 26/44 patients (59.1%) and more than 5 years for 18/44 patients (40.9%). The patients followed a gluten-free diet strictly (33/44, 75.0%) or semistrictly (11/44, 25.0%). The patients with untreated coeliac disease (15 patients; mean age 44.6 years; range 21–73 years; female : male ratio, 10 : 5) were enrolled from patients examined for coeliac disease at the Turku City Hospital and the District Hospital of Åboland, Turku, Finland. In both patient groups with coeliac disease the diagnosis was based on typical mucosal lesions found in the duodenal biopsy samples (subtotal or total villous atrophy), the presence of

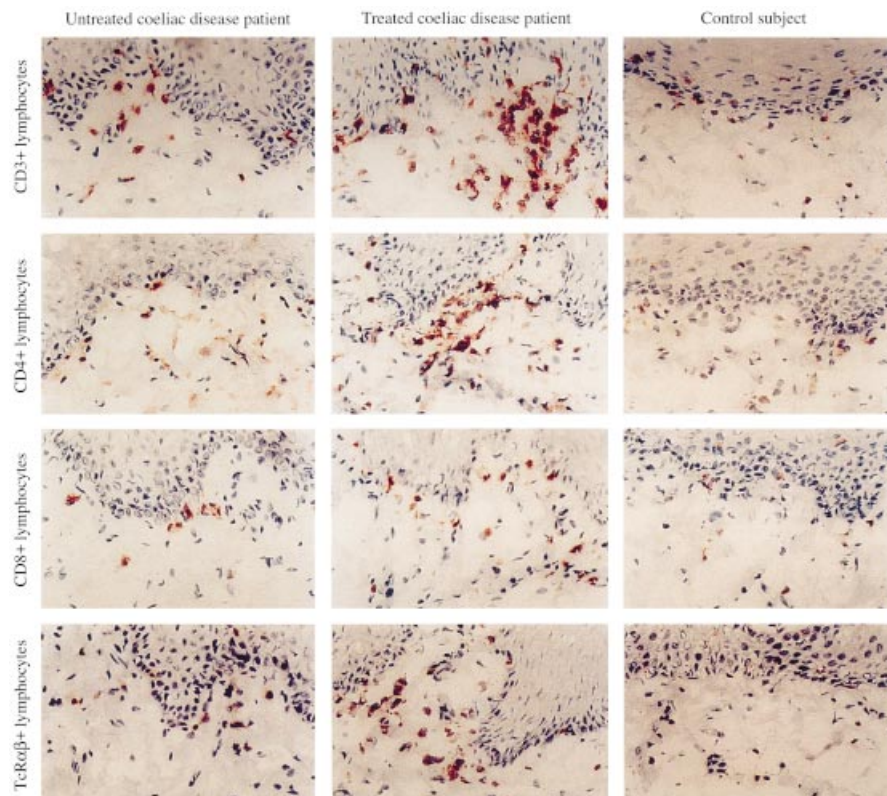


Fig. 1. Immunohistochemical staining of CD3⁺, CD4⁺, CD8⁺ and TCR $\alpha\beta$ in the oral mucosa. A panel of stainings in the buccal mucosa from patients with untreated coeliac disease and treated coeliac disease and from a control subject.

positive endomysial antibodies (Ab) and elevated values of antigliadin Ab in sera. Nineteen healthy volunteers (mean age 48.5 years; range 24–71 years; female : male ratio 12 : 7) served as controls for the coeliac disease patients. The study was approved by the Ethics Committee of the Faculty of Medicine, University of Turku, Finland.

Buccal samples. Buccal biopsy specimens were taken from normal-looking buccal mucosa below the occlusal line (linea alba) in the region of the second lower molar under local anaesthesia (Xylocain® adrenalin, ASTRA, Södertälje, Sweden). All specimens were divided into two parts. One half of each specimen was embedded in optimal cutting temperature compound (OCT compound, Tissue Tek; Miles Laboratories, Elkhart, IN, USA), snap-frozen in liquid nitrogen, and stored at -70°C until further analyzed. The other half was fixed in formalin (Baug & Bonsomer, Helsinki, Finland) and thereafter routinely processed into paraffin (ALGOWAX, Algolab, Espoo, Finland). All biopsy specimens taken were stained with a Periodic Acid-Schiff (PAS) staining method so that oral mucosal candidosis could be excluded.

Immunohistochemical staining. Mast cells were stained with toluidine blue. B lymphocytes were detected in paraffin-embedded sections using monoclonal antibodies (MoAb) to CD20 (Dako A/S, Glostrup, Denmark) and memory and/or activated T lymphocytes MoAbs to CD45RO (Dako A/S). Anti-CD45RO stains also a subpopulation of granulocytes, monocytes and dendritic cells. Paraffin sections were collected on organosilane-coated objective slides, deparaffinized in xylene and rehydrated in a graded series of ethanol. Endogenous peroxidase activity was blocked using 5% H_2O_2 in methanol for 5 min. The sections were then treated with 1.5% normal goat serum (Vector Laboratories, Burlingame, CA, USA) for 15 min. Incubation in the primary antibody (or in blocking serum) overnight at $+4^{\circ}\text{C}$ was followed by incubation in the secondary biotinylated antibody for 30 min and in the avidin-biotin-peroxidase complex (Vectastain Elite ABC Kit; Vector Laboratories) for 30 min. The immunoperoxidase reaction was developed using H_2O_2 and diaminobenzidine for 5 min. Finally, the sections were counterstained with haematoxylin. Formalin-fixed paraffin-embedded sections from tonsils and minor salivary glands of patients with Sjögren's syndrome (SS) were used as positive controls. Sections treated with blocking serum without primary antibodies served as negative controls. From the frozen specimens, 5- μm thick sections were cut and fixed in acetone for 10 min and in chloroform for 17 min at $+4^{\circ}\text{C}$. Endogenous peroxidase was blocked by incubation in 0.3% H_2O_2 in methanol for 30 min. After washing in phosphate-buffered saline (PBS) pH 7.4, the sections were incubated in MoAb for 1 hour. Bound primary Ab were then disclosed using the Vectastain Elite ABC kit (Vector Laboratories) according to the manufacturer's instructions using aminoethyl carbazole as chromogen and haematoxylin as counterstain. CD3⁺ intraepithelial lymphocytes were examined using MoAb Leu-4 (Becton Dickinson, San Jose, CA, USA), $\alpha\beta\text{TCR}$ molecules using MoAb βF1 (Endogen, Woburn, MA, USA) and $\gamma\delta\text{TCR}$ molecules using MoAb panTCRgd (Endogen). To detect CD4⁺ T cells MoAb Leu-3a+ 3b (Becton Dickinson) was used. For CD8⁺ cells we used MoAb Leu-2a (Becton-Dickinson). HLA DR expression was studied using MoAb HLA DR (Becton-Dickinson). CD25 (interleukin (IL)-2 receptor) positive cells were revealed with anti-CD25 antibody (Becton Dickinson). Ab were diluted with PBS containing 0.1% bovine serum albumin (BSA) as follows: MoAb Leu-4 1 : 15, βF1 1 : 40, panTCRgd 1 : 140, Leu-3a+ 3b 1 : 20, and Leu-2a 1 : 30, HLA DR 1 : 1000, and CD25 1 : 20.

Assessment of the immunopositivity. The total numbers of mast cells, B cells, CD45RO+ T cells (only small mononuclear lymphocytes were counted) and CD3⁺, CD4⁺, CD8⁺, TCR $\alpha\beta$ + and TCR $\gamma\delta$ + T cells

were light microscopically counted in the epithelium and in the lamina using an ocular graticule of 0.10 mm \times 0.10 mm at \times 400 magnification. Ten fields were counted in the buccal epithelium and lamina propria immediately below the basement membrane, and furthermore, for the presence of B cells, CD45RO+ T cells and mast cells, another 10 fields were counted deeper in the lamina propria (marked with B in the Tables). The results are given as cells/mm². To evaluate the HLA Class II expression, 10 fields were counted in the epithelium and lamina propria. HLA DR expression was interpreted as slight, slightly enhanced or enhanced and the results are given as percentages of the distribution according to the staining intensity. All microscopic examinations were carried out by the same investigator without prior knowledge of the origin of the biopsy specimen. Inter- and intraobserver variations were determined for the counts of CD45RO+ T cells.

Blood samples. Blood was collected from all subjects participating in the study within 1 week of the buccal biopsy procedure for checking serum endomysial antibody positivity [10].

Serum antibody determinations. Serum antiendomysial Ab (IgA class) were measured by an indirect immunofluorescence method using commercial monkey oesophagus as the substrate (BioSystems, Barcelona, Spain) [10].

Statistical analysis. Analyses were performed with the SAS Statistical Software Package for Windows (Release 6.12, 1996). The statistical tests used were the one and two sample *t*-test. Differences were considered significant when the *P*-value was less than 0.05.

RESULTS

All coeliac disease patients treated with a gluten-free diet except one (43/44), and all 19 controls were seronegative for endomysial Ab. All 15 patients with untreated coeliac disease had elevated endomysial Ab titres. The finding that all the patients with treated coeliac disease except one were negative for endomysial Ab reflects good mucosal recovery on a gluten-free diet. A comparison was also carried out between those patients adhering to a strict gluten-free diet and those on a semistrict gluten-free diet. Importantly, no significant differences were found between these groups in the numbers of inflammatory cell types in the oral mucosa (data not shown). We also looked for an effect of the duration of coeliac disease on inflammatory cell densities; however, no pattern of significance was observed between patients with a disease duration of less or more than 5 years. It has to be stressed that the buccal mucosal biopsy was always taken from clinically healthy-looking buccal mucosa.

Total counts of cell types in the epithelium and lamina propria of oral mucosa

The densities of intraepithelial CD45RO+ lymphocytes were significantly increased in treated coeliac disease patients as compared with untreated coeliac disease patients ($P < 0.001$) and controls ($P < 0.001$). CD45RO+ lymphocyte counts in the lamina propria of treated coeliac disease were increased, but however, did not reach statistically significant difference as compared with the other groups. Mast cells in the lamina propria were significantly increased ($P = 0.028$) in treated coeliac

Table 1. B cells, CD45RO+ T cells and mast cells of the oral mucosa in patients with untreated and treated coeliac disease (= CoD), and healthy controls

		B cells Cells/mm ²	T cells mean ± SD	Mast cells
<i>Epithelium</i>				
Untreated CoD patients, n = 15		0	14.3 ± 36.4	0
Treated CoD patients, n = 44		1.7 ± 7.9	102.7 ± 113.1*†	0.9 ± 4.9
Healthy controls, n = 19		0	24.9 ± 35.4	0
<i>Lamina propria</i>				
Untreated CoD patients, n = 15	A	0	172.8 ± 147.8	74.3 ± 48.6
	B	0	54.1 ± 84.0	88.8 ± 85.7
Treated CoD patients, n = 44	A	33.3 ± 136.7	441.4 ± 1045.9	120.1 ± 72.3‡
	B	20.8 ± 85.6	122.8 ± 265.1	104.8 ± 78.4
Healthy controls, n = 19	A	0	206.8 ± 212.4	97.2 ± 78.4
	B	0	45.1 ± 80.5	74.3 ± 48.6

Comparison of cell counts in epithelium: *, versus controls, $P < 0.001$; †, versus untreated, $P < 0.001$. Comparison of cell counts in lamina propria: ‡, versus untreated, $P = 0.029$. A = cells/mm² immediately below basement membrane; B = cells/mm² deeper in lamina propria. Stainings were made using paraffin-embedded sections. Two sample *t*-test.

disease patients as compared to untreated patients. Interestingly, B cells were rare and they were observed only in the oral mucosa of treated coeliac disease patients (Table 1).

The mean counts of CD3⁺, CD4⁺ and CD8⁺ lymphocytes are summarized in Table 2. The numbers of CD3⁺, CD4⁺ and CD8⁺ T cells in the surface epithelium were similar in all study groups but were increased in the lamina propria of the

oral mucosa of the treated coeliac disease patients when compared with the untreated coeliac disease patients and the controls. Besides the increase in CD3⁺ cells ($P = 0.019$ versus controls and $P = 0.004$ versus untreated coeliac disease patients), there was a considerable increase in CD4⁺ T cells ($P = 0.042$ versus controls and $P = 0.025$ versus untreated coeliac disease patients). The numbers of CD8⁺ cells were

Table 2. T-cell subsets and HLA DR expression of the oral mucosa in patients with untreated and treated coeliac disease (= CoD), and healthy controls

	Cells/mm ² ; mean ± SD					HLA DR staining		
	CD3 ⁺	CD4 ⁺	CD8 ⁺	Alpha/beta+	gamma/delta+	slight	enhanced	enhanced
<i>Epithelium</i>								
Untreated CoD patients, n = 13/15	157.1 ± 39.2	63.4 ± 34.1	95.7 ± 29.0	67.2 ± 37.0	0	27.3%	61.3%	11.4%
Treated CoD patients, n = 44	152.2 ± 86.1	89.0 ± 68.9	98.1 ± 84.0	78.9 ± 48.6	1.1 ± 4.3	40.0%	50.0%	10.0%
Healthy controls, n = 19	159 ± 168.2	84.2 ± 83.5	103 ± 115.4	116.2 ± 118.6	1.2 ± 3.2	42.1%	52.6%	5.3%
<i>Lamina propria</i>								
Untreated CoD patients, n = 13/15	232.7 ± 57.1	362.1 ± 206.8	160.8 ± 87.4	162.1 ± 26.7	0	40.9%	43.3%	15.8%
Treated CoD patients, n = 44	967.3 ± 1510.6*†	982.8 ± 1624.5‡§	295.3 ± 373.5	480.4 ± 569.7	7.2 ± 20.7¶**	70.0%	20.0%	10.0%
Healthy controls, n = 19	336.8 ± 336.4	396.0 ± 436.5	154.6 ± 156.6	342.1 ± 332.3	0	57.9%	26.3%	15.8%

Comparison of cell counts in epithelium: No significant differences. Comparison of cell counts in lamina propria:*, versus controls, $P = 0.019$; †, versus untreated, $P = 0.004$; ‡, versus controls, $P = 0.042$; §, versus untreated, $P = 0.025$; ¶, versus controls, $P = 0.035$; **, versus untreated, $P = 0.035$. Stainings were made using frozen sections. Two sample *t*-test.

increased in the lamina propria of oral mucosa in the treated coeliac disease patients, but the increase did not reach statistical significance.

As shown in Table 2, the numbers of TCR $\alpha\beta$ + and TCR $\gamma\delta$ + T cells in the lamina propria (mean \pm SD; 480.4 ± 569.7 and 7.2 ± 20.7 cells/mm²) were higher in the treated coeliac disease patients than in the untreated coeliac disease patients (mean \pm SD; 162.1 ± 26.7 and 0 cells/mm²) or in the controls (mean \pm SD; 342.1 ± 332.3 and 0 cells/mm²). The counts of TCR $\gamma\delta$ + T cells in the lamina propria of the treated coeliac disease patients were small compared with the counts of $\alpha\beta$ TCR+ T cells. The numbers of $\alpha\beta$ and $\gamma\delta$ TCR+ cells in the surface epithelium did not differ in the patient groups studied and the controls. Interestingly, TCR $\gamma\delta$ + T cells were not detected intraepithelially or in the lamina propria in the untreated coeliac disease patients.

The counts of CD3⁺ T cells in the lamina propria were significantly higher than those of $\alpha\beta$ + T cells both in treated coeliac disease patients ($P = 0.013$) and in untreated coeliac disease patients ($P = 0.014$) (P -values not shown in Table 2). As the counts of TCR $\gamma\delta$ T cells were low both in patients with untreated and treated coeliac disease, a large proportion of T cells bore no TCR. In contrast, in the lamina propria of the controls, the counts of CD3⁺ T cells were similar to those of TCR $\alpha\beta$ + cells.

HLA DR expression in epithelium and lamina propria of oral mucosa

The positivity of HLA DR was divided into three categories (slight, slightly enhanced and enhanced). In this study, the expression of the HLA DR of oral mucosa was similar in the coeliac disease patients and the controls. Thus, neither untreated nor treated coeliac disease had any clear effect on the expression of HLA DR in the oral mucosa.

DISCUSSION

We now show for the first time that oral mucosal infiltrations of T-cell subsets are not increased in untreated coeliac disease patients as one might expect. On the contrary, treated coeliac disease patients showed significantly increased numbers of inflammatory cells in the oral mucosa. There was no difference in numbers of T cells or T-cell subsets in the patients on a strict or semistrict gluten-free diet. Importantly, all but one of the 44 patients with treated coeliac disease were negative for serum endomysial Ab, a sensitive and specific indicator of mucosal damage in coeliac disease [10–12].

In the present study, e.g. the numbers of CD45RO+ lymphocytes counted in paraffin sections and CD3⁺ T cells enumerated in frozen sections showed slight differences. T lymphocytes were stained using with CD45RO+ specific Ab, which mainly stains memory/activated T cells, but also some granulocytes, monocytes and dendritic cells (DC). Naïve or resting T lymphocytes are not stained with the used CD45RO+

Ab and therefore, it stains only a subpopulation of CD3⁺ lymphocytes. T lymphocytes can be, however, easily distinguished from monocytes and macrophages by microscopic appearance, and they were not taken into account when the CD45RO+ cells were enumerated. Another explanation for the slight differences obtained in the numbers of CD45RO+ T cells and CD3⁺ T cells is that CD45RO+ lymphocytes were stained on the paraffin sections and other T cells on the frozen sections. Although the stainings should have been optimally made on the same part of the biopsy specimen, this was not possible for practical reasons. There are also other sources of variation in the enumeration of the immunohistochemical examinations. If the biopsy specimen is not cut vertically, it may cause difficulties in the interpretation. In addition, if the amount of cells to be counted is very abundant the counting is difficult to perform reliably. These aforementioned reasons can explain the differences between the CD45RO+ and CD3⁺ lymphocyte counts.

In coeliac disease, TCR $\alpha\beta$ + CD8⁺ T cells (70–90% of total T cells) are markedly increased in the small intestinal epithelium and are admixed with a disproportionately enlarged TCR $\gamma\delta$ + CD8-subset of T cells [13–15]. The lamina propria of the jejunal mucosa, on the other hand, contains increased numbers of TCR $\alpha\beta$ + CD4⁺ T cells [4, 16], while only occasional TCR $\gamma\delta$ + cells have been observed [17]. In the present study the significant increase in the numbers of CD3⁺ cells in the lamina propria of the oral mucosa seems to be an outcome of the expansion of T cells with no TCR. In fact, all T cells detected to bear a TCR carried the $\alpha\beta$ +TCR, and there was no tendency for a recruitment of TCR $\gamma\delta$ + T cells. It is possible that the recruited cells could in part be so-called natural killer (NK) cells. Phillips and coworkers have shown that NK cells express cytoplasmic CD3 [18]. The role of NK cells in the oral mucosa of treated coeliac disease patients remains elusive. We conclude that in contrast to the small intestinal mucosa, the measurement of TCR $\gamma\delta$ + T cells in the oral mucosa is of no diagnostic value in coeliac disease, and TCR $\gamma\delta$ + T cells might not be involved in the oral mucosal pathology of coeliac disease patients.

In coeliac disease, the stratified oral epithelium might not behave similarly to the epithelium of the gut, which is composed of a single layer of epithelial cells. In the small intestinal mucosa, the increase of T cells occurs in untreated coeliac disease, and the density of these cells decreases on a gluten-free diet. In the rectal mucosa of untreated coeliac disease patients, CD3⁺, but also TCR $\gamma\delta$ + T cells, plasma cells and mast cells are increased in number and these changes were reversed, with the sole exception of mast cells, by dietary treatment [19]. Again, oral mucosal findings were not in agreement with the changes observed in the rectal mucosa. In our patients with treated coeliac disease, a significant increase of oral mucosal total CD3⁺ T cells and CD4⁺ cells was seen, although the patients had been on a strict gluten-free diet. Also the number of mast cells in the oral mucosal lamina propria of treated coeliac disease patients was increased, but their role remains unclear.

The number of B cells was very low in the oral mucosa of treated coeliac disease patients and they were not found at all in untreated coeliac disease patients or in the controls.

Gastrointestinal mucosa-associated lymphoid tissue (GALT) consists of intestinal Peyer's patches, lymphocytes of the lamina propria, intraepithelial lymphocytes, and the gastric mucosal immunosurveillance system [20–23]. Nothing is known about active homing molecules in the oral mucosa, but being at the interface of the gut and skin the immune system of the oral mucosa may be a mixture of both immune systems and homing mechanisms. Generally, in coeliac disease patients, a lifelong immunological memory for gluten hypersensitivity is perpetuated, which is stored in memory T cells [3]. In patients with untreated coeliac disease a normal diet containing large amounts of gluten induces a sequestration of lymphocytes to the intestinal mucosa. This may result in low numbers of T cells in other mucosal tissues as in the oral mucosa. After the introduction of a gluten-free diet (GFD), the actual inflammation in the intestine settles down and memory T cells home to the sites of the mucosal immune system where they are needed [24].

It is difficult to explain why T lymphocytes are increased in the oral mucosa of treated coeliac disease patients. It is well established that extravasation of specific T cells to a tissue does not depend on antigen, but the arrest and local proliferation may. Thus, as a hypothesis, we suggest that even minute amounts of gluten may activate these memory T lymphocytes emigrated to the oral mucosa to increase in numbers, and this could explain the increased oral mucosal inflammation despite a gluten-free diet. Such minute amounts of gluten can exist in foodstuffs as remains. Furthermore, many products reported to be gluten-free may be contaminated with gluten, or as is the case with wheat starch products. The latter are allowed to include proteins up to 5%. Our fingers may be contaminated with gluten, or gluten may be present in the air as flour dust. Furthermore, contamination with gluten-containing cereals in products gluten-free by nature (e.g. maize, rice, soybean) has been detected and involves further risks for small gluten intake for coeliac disease patients [25]. In a recent study, elevated serum antigliadin Ab were observed in millers and bakers as the result of high exposure to flour dust antigens present in the air [26]. However, the extent of gluten repeatedly activating oral mucosal T cells may not be large enough to induce small intestinal deterioration. Thus, the increased T-cell subpopulation densities in the oral mucosa might represent a late immune response of coeliac disease patients.

CONCLUSIONS

In conclusion, we clearly show that the oral mucosa can not be used as a marker of untreated coeliac disease. Furthermore, the total number of oral mucosal TCR $\gamma\delta$ + T cells was very small both in untreated and treated coeliac disease patients, and we could not show any evidence of active recruitment of TCR $\gamma\delta$ + T cells in the oral mucosa in coeliac disease. T-cell subsets, especially CD3⁺ lymphocytes are markedly increased

in the oral mucosa of patients with treated coeliac disease. This phenomenon seems to take on a chronic character with time despite strict adherence to a gluten-free diet. We assume that, in the oral cavity of treated coeliac disease patients, a late immune response may occur to minute amounts of gluten, leading to increases in gluten-responsive T cells. This last observation requires much further research, e.g. sequential biopsies in the same patients over a longer period of time might be required.

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