Histomorphology of healthy oral mucosa in untreated celiac patients: unexpected association with spongiosis

G. Campisi[1], D. Compilato[1], G. Iacono[2], E. Maresi[3], C. Di Liberto[1], V. Di Marco[4], G. Di Fede[5], A. Craxì[4], A. Carroccio[6]

[1]Department of Oral Sciences, University Hospital of Palermo, Palermo, Italy; [2]First Division of Pediatric Gastroenterology, "G. Di Cristina" Hospital of Palermo, Palermo, Italy; [3]Department of Human Pathology, University Hospital of Palermo, Palermo, Italy; [4]Gastroenterology and Hepatology Unit, University Hospital of Palermo, Palermo, Italy; [5]Department of Medical Oncology, University Hospital of Palermo, Palermo, Italy; [6]Department of Internal Medicine, University Hospital of Palermo, Palermo, Italy

BACKGROUND: The jejunal mucosa is the major site involved in celiac disease, but modifications have also been found in the gastric, rectal and esophageal mucosa. Few studies have focused on the histomorphological features of the oral mucosa in celiac disease patients. Our objectives were: (i) to assess the presence, quality and intensity of lymphocytic infiltrate in clinically healthy oral mucosa and its relation to celiac disease severity (villous height to crypt depth ratio); and (ii) to detect any other histological features connected to celiac disease.
METHODS: Twenty-one untreated celiac disease patients (age range 13–68 years) with clinically healthy oral mucosa were enrolled and compared with 14 controls. Intestinal and oral biopsies were carried out and specimens were evaluated after staining with hematoxylin and eosin.
RESULTS: Intra-epithelial lymphocyte B and T infiltrates of the oral mucosa were found to be similar in both groups; likewise, intensity of the lymphocytic infiltrate in the lamina propria was similar in both groups and was not related to intestinal damage; important signs of spongiosis were found to be more significantly present in celiac disease patients compared with controls ($P = 0.0002$).
CONCLUSIONS: Our study showed that the healthy oral mucosa of untreated patients does not reflect the intestinal damage by celiac disease, but it is unexpectedly affected by spongiosis, as being detected for the first time in the literature. This latter feature could be related to gliadin ingestion and could contribute to explain the higher susceptibility of celiac disease patients to suffering from oral mucosa lesions.
J Oral Pathol Med (2009) 38: 34–41

Keywords: celiac disease; lymphocytic infiltrate; oral mucosa; spongiosis

Introduction

Celiac disease (CD), the most common food-sensitive enteropathy in humans, can be defined as a chronic inflammatory intestinal disease that affects the small intestinal mucosa, triggered by the ingestion of gluten-containing cereals (i.e., wheat, barley, rye) in genetically predisposed subjects (1–3). Its prevalence is estimated in the range of 1:85–1:300 both in Europe and in the USA (4–11). It is known that CD results from an inappropriate T cell-mediated immune response against ingested gluten (1, 12, 13), in fact the activation of the intestinal intraepithelial lymphocytes and lymphocytes in the lamina propria produces peculiar damages of the small intestinal mucosa observed in the intestinal biopsy specimens. Normal small intestinal mucosa exhibits digitate villi, leaf forms and ridges. The villi vary in size, shape, and height but their heights are usually three times more than their widths. The classic histological intestinal lesion of a patient with untreated CD is characterized by villous atrophy (partial, subtotal or total) with crypt hyperplasia (14), increase in lamina propria and intraepithelial lymphocytes (the normal gut contains 10–30 intraepithelial lymphocytes/100 enterocytes), extensive surface cell damage and inflammatory infiltration of the lamina propria (15) (Fig. 1).

Although the small intestinal mucosa represents the main site of the gut involved in CD, other mucosal surfaces belonging to the gastrointestinal (GI) tract and to the gut-associated lymphoid tissue (GALT) can also be involved (16–18). In fact, changes have been observed in the gastric (19, 20), terminal ileal (21) and rectal mucosa (16, 22–26); rectal mucosa, furthermore, has also been used for gluten challenges (22, 27–31). Finally, esophageal involvement has also been shown in patients with untreated CD (19, 32). All this demonstrates that gluten-driven T-cell activation is not restricted to the proximal part of the intestine but is present in the whole intestine.

A site which could be studied less invasively is the mouth, as it is the first part of the GI system and a part
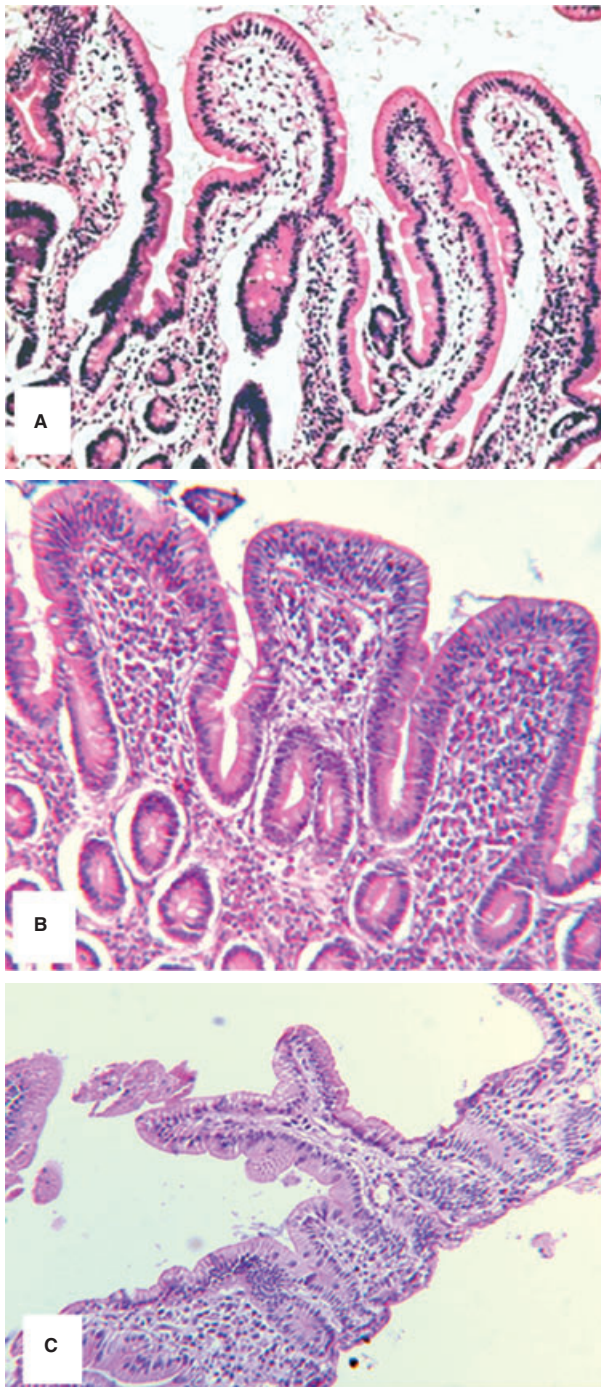
Figure 1 Histological features of small intestinal mucosa in patients with untreated CD. (A) Normal mucosa. (B) Partial villous atrophy. (C) Total villous atrophy.

of GALT; in fact, it is well known that the oral mucosa can be affected by clinical manifestations in patients with CD (33–41). Furthermore, it has been shown that inflammatory changes occur after oral supramucosal application and submucosal injection of gliadin in the oral mucosa of CD patients (42–44) and a recent study reported that the oral mucosa contributes to anti-endomysial antibodies (EMA) and anti-tissue transglutaminase (tTG) antibodies production in untreated CD

patients (45). However, currently only few data exist on the histological aspects of oral mucosa in patients with CD.

Hence, the major aim of this study was to study the clinically healthy oral mucosa of CD patients: (i) to assess the presence, quality (B/T lymphocytes) and intensity of lymphocytic infiltration and its relation to the severity of the intestinal histological damage; and (ii) to detect any other histological aspect which could be linked to CD.

Materials and methods

The test group consisted of 21 newly diagnosed, and untreated CD patients (15 females and 6 males, median age 36 ± 14 years; range 13–68 years) consecutively enrolled in a secondary hospital setting, between June 2004 and May 2005.

During the same period, 14 subjects (10 females and 4 males, median age 39 ± 15 years; range 12–66 years), matched for age and gender, were consecutively recruited from patients with symptoms compatible with CD diagnosis (weight loss, failure to thrive, anemia, chronic diarrhea, abdominal pain, dyspepsia, gastro-esophageal reflux and abdominal meteorism), but with negative serum assays for CD diagnosis. All underwent eso-phago-gastro-duodenoscopy (EGDS) with duodenal histological examination. All showed normal histology of the intestinal mucosa and were considered control subjects.

Celiac disease diagnosis was based on positive serology [IgA and/or IgG anti-tTG, IgA and/or IgG EMA] together with positive histological evidence of villous atrophy with crypt hyperplasia and increase in intraepithelial lymphocytes, and the disappearance of symptoms and normalization of serum anti-tTG and/or EMA after a gluten-free diet (GFD) was imposed (46).

Test and control patients were recruited after a careful clinical examination of the entire oral cavity and subjects showing soft tissue lesions (e.g., aphthous-like ulcers, non-specific atrophic glossitis, mucosal erythema) were excluded from this study.

Informed consent was obtained from all of the patients involved in the study or from their parents in the case of pediatric patients.

Intestinal and oral biopsies, histological and immunohistochemical evaluation
Following general anesthesia (in children) or conscious sedation with meperidine/pethidine 1 mg/kg i.v. and midazolam 0.05–0.1 mg/kg i.v. (in adults), EGDS were performed with standard pediatric or adult video-colonoscopes (Olympus, Milan, Italy). At least four duodenal biopsy samples were obtained from each patient.

Both 21 untreated CD patients and 14 controls underwent oral mucosal biopsy. Biopsy specimens were taken at the same time as the EGDS in the children and under local anesthesia (Xylocaine® 2%, adrenaline 1:100.000) in adults. The specimens were always taken by two of the authors (DC and CDL)

from a clinically healthy buccal mucosa adjacent to the right second molar tooth region, beneath the occlusal line. The biopsies were performed by punching (6 mm in diameter punch biopsy) or by incision with cold knife.

The intestinal and oral biopsy specimens were immediately fixed in 10% neutral-buffered formalin for 2 h, washed in water for 1 h, dehydrated in graded ethanol (60%, 80%, 90%, 95% and, 100%) and after permeation in xylene, embedded in paraffin using the standard procedures. The formalin-fixed, paraffin-embedded samples were cut into 5-μm-thick sections on a microtome with a disposable blade. The sections of the intestinal mucosa were stained with hematoxylin and eosin (H&E) for standard evaluation. From each specimen of the oral mucosa, three sections were obtained: one conventionally stained with hematoxylin-and-eosin and the other two treated with 4 KB5 monoclonal antibodies against-CD45RA and with UCHL1 monoclonal antibodies against-CD45RO (Dako, Milan, Italy) to evaluate lymphocyte infiltrates B and T, respectively.

Intestinal histological specimens were defined compatible with the clinical diagnosis of CD when there was both intraepithelial lymphocytosis (at least 40 lymphocytes/100 enterocytes) (47) and a villous height to crypt depth ratio (V/C) < 2.5. Furthermore, based on this ratio, a CD histological grading was defined: Grade 1 = V/C 2–2.5; Grade 2 = V/C 1–2; Grade 3 = V/C 1–0.5; and Grade 4 V/C < 0.5 (48, 49).

All oral biopsy slides were examined blindly by one of the authors (EM). $CD3^+$ intraepithelial lymphocytes were examined using mAb Leu-4 (Becton Dickinson, San Jose, CA). B lymphocytes were detected in paraffin-embedded sections using monoclonal antibodies to CD20 (Dako a/s, Glostrup, Denmark) and T lymphocytes with monoclonal antibodies to CD45RO (Dako a/s).

The densities of $CD3^+$, $CD20^+$ and $CD45RO^+$ cells were light microscopically counted in the epithelium and in the *lamina propria* using an ocular graticule of 0.10×0.10 mm at ×400 magnification. Ten fields were counted in the buccal epithelium and lamina propria immediately below the basement membrane. The results were given as cells/mm². The intensity of the *lamina propria* lymphocytic infiltrate in the H&E stainings was arbitrarily graded as none (0), low (1), moderate (2) or severe (3).

Furthermore, the presence of a spongiotic tissue reaction was assessed. Spongiotic reaction was defined as the presence of intraepidermal and intercellular oedema and labeled 'spongiosis'.

Statistical analysis
Data were analyzed by means of STATVIEW for Windows (SAS Inc v. 5.0.1, Cary, NC, USA). To measure the association level, odds ratio (OR) and the 95% corresponding test-based confidence interval (CI) were calculated. Chi-square test was used to assess statistical differences between categorical variables. The Mann–Whitney U-test and Spearman rank correlation coefficient (Spearman's rho) were used to calculate differences between non-parametric continuous variables. Student's t test was used for parametric continuous variables. In all of the evaluations, P-values ≤0.05 were considered statistically significant.

Results

Table 1 shows the lymphocyte (B and T) counts in the oral mucosa of the 21 CD patients and of the 14 controls. No differences were observed in $CD3^+$ count or B- and T-lymphocyte counts either in the epithelium or in the *lamina propria* of the oral mucosa in CD patients and in controls, although in the *lamina propria* of the CD patients there was a trend towards a higher number of $CD3^+$ and CD45RO lymphocytes.

Furthermore, there was no relationship between the intensity of lymphocytic infiltration in the oral mucosa and severity of intestinal mucosal damage, evaluated as villi/crypts ratio ($r = 1.03$, $P = 0.8$) (Table 2).

A very frequent histomorphological finding was spongiosis of the oral mucosal epithelium, observed in 19/21 (90%) of CD patients and only in 4/14 (29%) of controls [$P = 0.0002$; $\chi^2 = 14.29$; OR = 23.75 (95%CI = 3.69:152.9)] (Fig. 2). This spongiotic reaction was characterized by the presence of intraepidermal and intercellular oedema; intercellular spaces were widened with an elongation of the intercellular bridges. The foci of spongiosis were also characterized by a mild lymphocytic infiltrate, but not by the presence of eosinophils or neutrophils. These lesions were exclusively microscopic without any corresponding identifiable vesicles on the oral mucosal surface (Figs 3–4).

Discussion

Although histological and clinical features of the small intestinal mucosa in CD are well known, the involvement of the other mucosal surfaces belonging to the GI tract and to the GALT has been neglected. It has been demonstrated that in CD patients, gluten-driven T-cell activation is not restricted to the proximal part of the intestine, but is present in the whole intestine. In fact, the dissemination of the immune effector cells involves very distant sites of the GALT including the oral and rectal mucosa (17), the two extremities of the GI tract.

Table 1 Number of T- and B-lymphocytes in the oral epithelium and in the lamina propria of 21 untreated CD patients and of 14 controls (intra-epithelial lymphocytes were counted as cells/mm²; lamina propria lymphocytes as cells × 100/mm²)

|	CD patients	Controls
Intra-epithelial		
$CD3^+$	152 ± 121	168 ± 110
B-lymphocytes	43 ± 42	38 ± 45
T-lymphocytes	121 ± 102	131 ± 98
Lamina propria		
$CD3^+$	5.1 ± 4.7	3.1 ± 3.8
B-lymphocytes	0.8 ± 0.9	0.6 ± 0.7
T-lymphocytes	4.5 ± 2.9	3.8 ± 3.9

Data are given as mean ± SD.

Table 2 Relationship between the intensity of lamina propria lymphocyte infiltration in the oral mucosa and the severity of intestinal mucosa damage

	Intestinal CD histological grading				Number of
	Grade 1	Grade 2	Grade 3	Grade 4	CD patients
Grading of oral lymphocytic infiltrate					
None	0	0	1	5	6
Low	0	2	3	9	14
Moderate	0	0	0	0	0
Intense	0	0	0	1	1

Histological changes have already been shown in the gastric (19, 20), terminal ileal (21), rectal mucosa (16, 22, 26), and esophageal mucosa (19, 32) of CD patients.

Although it is widely reported that in CD patients, the mouth can also be affected by several clinical manifestations such as recurrent aphthous stomatitis (RAS) (34, 38, 40, 50), dental enamel defects (39, 51–54) and non-specific atrophic glossitis (41), data about histological features of clinically healthy oral mucosa in patients with CD are few and often contrasting (41, 42, 55).

The healthy mucosa of the mouth is quite different from that of the rest of the GI tract; the oral mucosa of the lips, buccal, and floor areas are covered by a non-keratinized stratified epithelium, while the lining of the stomach and small and large intestine consists of a simple epithelium composed of only a single layer of cells (56). Although the structure of the oral mucosa differs from that of the bowel from an immunological point of view, the mouth is situated at the beginning of the GI system and, as part of it, is considered to belong to the GALT. Consequently, after antigen activation, proliferation and partial differentiation in the GALT, memory B cells and T cells migrate to other parts of the GALT, including the oral mucosa lymphoid tissues (17). There, these cells, in sensitized CD patients, may easily react to gluten and its peptides; in fact, more recent findings have suggested that the oral mucosa of CD patients could be useful to evaluate response to a gluten-challenge (42).
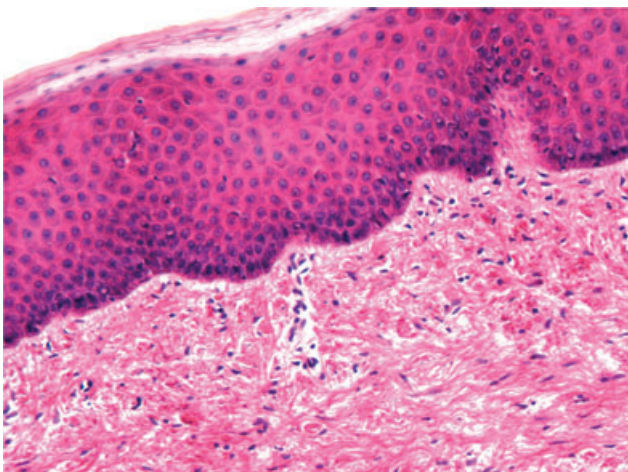
Figure 2 Normal oral mucosa. Absence of spongiosis in the oral epithelium.

Based on these considerations, we expected the healthy oral mucosa of untreated CD patients to behave in a similar way to the small bowel mucosa. However, our results did not confirm our initial hypothesis and we did not find any significant presence of B and T lymphocytes infiltrate or any relationship with severity of CD in the oral mucosa of our study group.

These data are in agreement with a study performed in Finland in which untreated CD patients did not differ from controls in terms of T cell number in the buccal mucosa (55). The behavior of the oral mucosa of our patients with untreated CD did not mirror that of the small intestinal mucosa in which an increase of T cells generally occurs and the density of these cells decreases on a GFD. An explanation of this result might be that in patients with untreated CD, a gluten-containing diet induces a sequestration of lymphocytes to the intestinal mucosa and hence a 'concentration' of the inflammatory cells with greater mucosal damage at this site (57). This may result in a low intensity of lymphocytic infiltrate in other mucosal tissues belonging to the GALT, including the oral mucosa (41, 58).

The novel finding of our research is the detection of 'spongiosis' in the biopsy samples of the clinically healthy oral mucosa of untreated CD patients. We found epithelial spongiosis in almost all the study group (90%) with a crude OR of about 23.

In general, spongiotic tissue is characterized by the presence of intracellular and intercellular oedema. Indeed, spongiosis is considered only a histopathological concept and not as a clinical entity, although several diseases present such a tissue reaction (59).

Spongiotic reaction has been found in several skin lesions (60–64) and in some oral diseases, such as oral melanoacanthosis (melanoacanthoma) (65), oral psoriasis (66), allergic contact stomatitis (67), plasma cell gingivitis, intra-oral fixed drug eruption, leukoedema and white sponge nevus (68). In most of these oral lesions, the pathogenetic mechanism involved in the collection of the intraepithelial fluid is not clear and remains to be elucidated: spongiosis could be caused by extravasations of fluids from blood vessels located in the *lamina propria* or by the presence of an osmotic gradient developed towards the epithelium, drawing fluid into it subsequent to various immunological reactions (59).

Spongiosis is also a histopathological feature found in the epithelium at the margin of an aphthous ulcer (68); moreover, the pre-ulcerative stage in the pathogenesis of RAS is characterized by oedema of the epithelium and keratinocyte vacuolization (69), and therefore by spongiosis.

However, as several conditions, like the presence of mucopolysaccharidic materials may simulate spongiosis, we demonstrate that this histomorphologic feature is not associated with the accumulation of PAS-positive substances (e.g., acid mucopolysaccharides) within the cytoplasm of the keratinocytes with spongiotic aspect (Fig. 5).

We underline that our finding of massive spongiosis was found in the otherwise healthy oral epithelium of
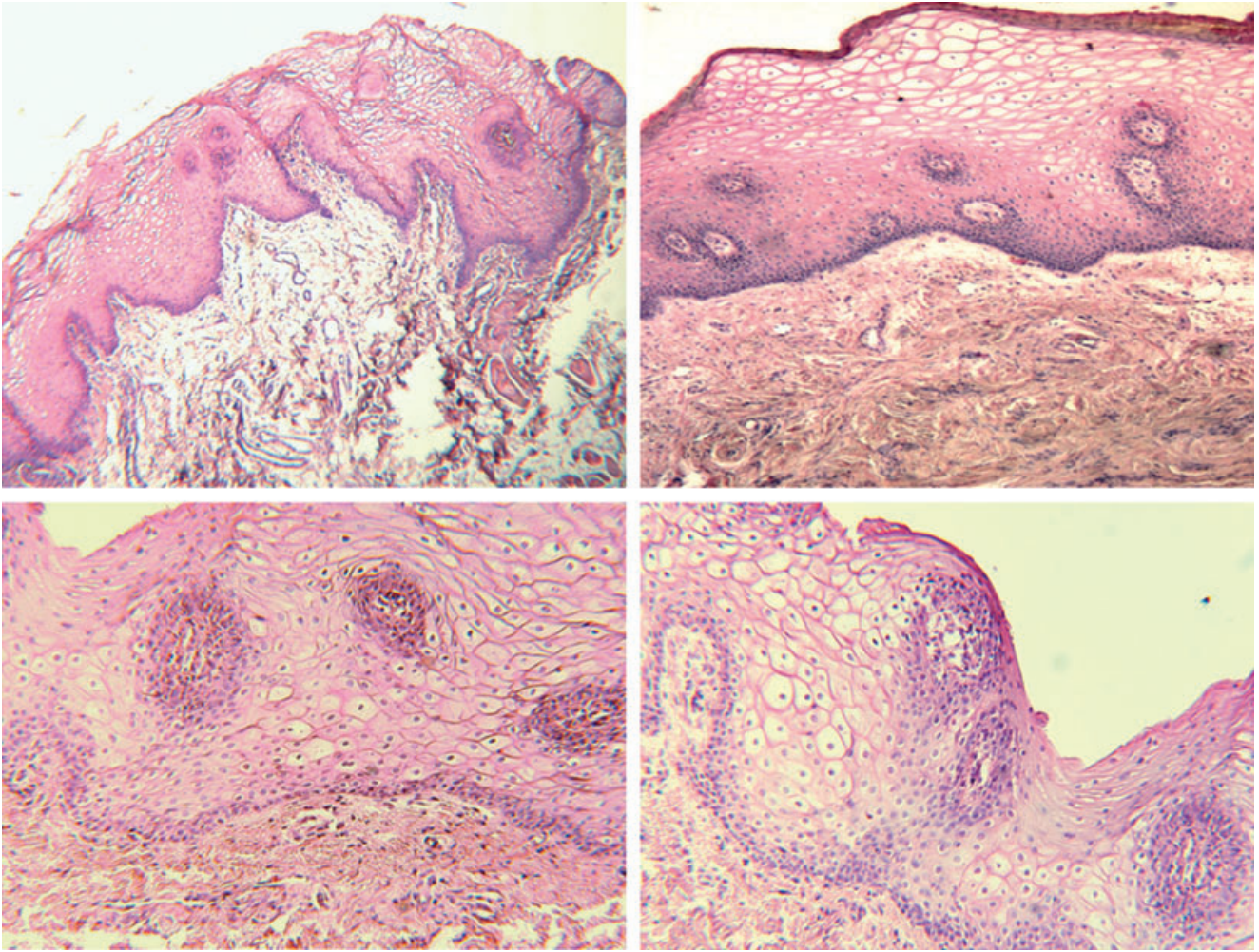
Figure 3 Histological features of healthy oral mucosa in different celiac patients. Diffuse distension and vacuolation (spongiosis) of the intermediate and superficial acanthocytes and their overlapping.
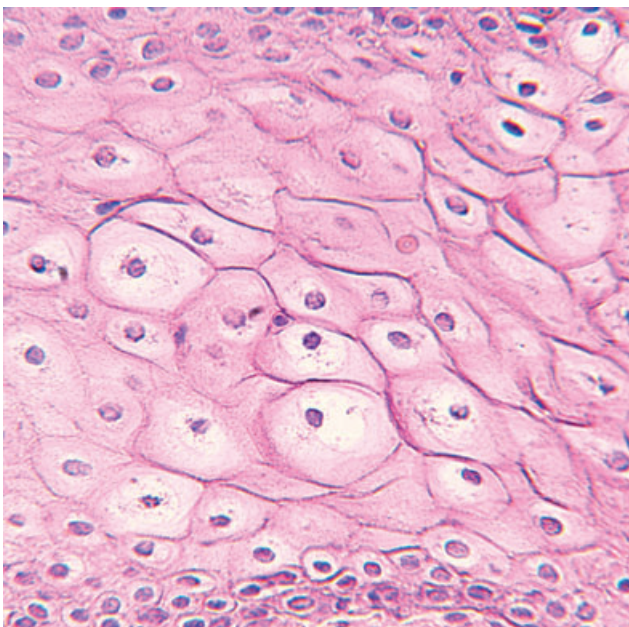

Figure 4 Marked overlapping of the acanthocytes.

CD patients. Noteworthy, this finding may support the hypothesis that spongiosis represents histological evidence or a key of the well-known higher susceptibility of these patients to suffering from RAS. The continuous contact of the gluten-containing foods with the oral mucosa could provoke epithelial spongiotic changes, characterized first by intracellular oedema and then by intercellular oedema with elongation of the intercellular bridges. The increase of intercellular oedema leads to the formation of intraepithelial micro-vesicles and thereafter breaking off to form ulcer. According to this theory, patients not adhering to GFD would be more likely to suffer from RAS, as actually verified by our research group (70).

The lack of lymphocytes infiltration of the oral mucosa cannot exclude that other inflammatory mechanisms, cells and mediators play a role in determining an inflammatory condition that determines the onset of spongiosis.

A further datum comes from a recent study (71) in which authors showed that the clinically healthy oral mucosa of patients who underwent allogeneic hematopoietic stem cell transplantation presents the same
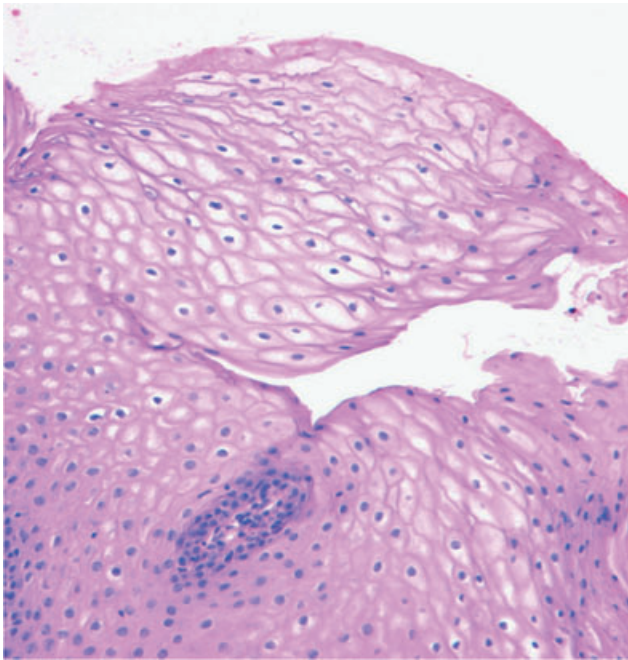
Figure 5 Absence of PAS-positive substances (e.g., acid mucopolysaccharides) within the cytoplasm of the keratinocytes with spongiotic aspect.

histological changes as those observed in biopsy specimens of patients with clinical evidence of oral chronic graft-versus-host disease (c-GVHD); in this case, a basal cell hydropic degeneration resembling the spongiosis reported by us was observed.

This latter study, as well as ours, proposes that oral biopsy could represent an important and useful diagnostic method for predicting the clinical onset of oral lesions in patients with some systemic diseases, such as CD or c-GVHD, especially if we consider that the oral cavity represents a site with less invasiveness and with an easy access.

In conclusion, our findings confirm that the oral mucosa of untreated CD cannot be considered a counterpart of the jejunal mucosa, at least in terms of B and T lymphocyte infiltrate.

The finding of oral mucosal spongiosis, to the best of our knowledge, is the first reported for CD; its meaning is not definitively explained, but it could provide the well-known susceptibility of CD patients to RAS onset. In these patients with untreated CD, oral mucosal spongiosis may be related to histological phenomena induced by gliadin ingestion. Hence, future aims could be to demonstrate whether oral spongiosis is a reliable sign of naïve CD, and whether it should be used as a marker of untreated CD, an indicator of non-adherence to GFD or a condition present despite strict adherence to GFD.

Conflict of interest

Professor Giuseppina Campisi is the guarantor of the paper. All the authors have declared that none of them

References

1. Green PH, Jabri B. Coeliac disease. *Lancet* 2003; **362**: 383–91.
2. Catassi C, Fasano A. New developments in childhood celiac disease. *Curr Gastroenterol Rep* 2002; **4**: 238–43.
3. Cataldo F, Montalto G. Celiac disease in the developing countries: a new and challenging public health problem. *World J Gastroenterol* 2007; **13**: 2153–9.
4. Catassi C, Ratsch IM, Fabiani E, et al. High prevalence of undiagnosed coeliac disease in 5280 Italian students screened by antigliadin antibodies. *Acta Paediatr* 1995; **84**: 672–6.
5. Kolho KL, Farkkila MA, Savilahti E. Undiagnosed coeliac disease is common in Finnish adults. *Scand J Gastroenterol* 1998; **33**: 1280–3.
6. Not T, Horvath K, Hill ID, et al. Celiac disease risk in the USA: high prevalence of antiendomysium antibodies in healthy blood donors. *Scand J Gastroenterol* 1998; **33**: 494–8.
7. Korponay-Szabo IR, Kovacs JB, Czinner A, Goracz G, Vamos A, Szabo T. High prevalence of silent celiac disease in preschool children screened with IgA/IgG antiendomysium antibodies. *J Pediatr Gastroenterol Nutr* 1999; **28**: 26–30.
8. Carlsson AK, Axelsson IE, Borulf SK, Bredberg AC, Ivarsson SA. Serological screening for celiac disease in healthy 2.5-year-old children in Sweden. *Pediatrics* 2001; **107**: 42–5.
9. Hill ID, Bhatnagar S, Cameron DJ, et al. Celiac disease: Working Group Report of the First World Congress of Pediatric Gastroenterology, Hepatology, and Nutrition. *J Pediatr Gastroenterol Nutr* 2002; **35** (Suppl. 2): S78–88.
10. Fasano A, Berti I, Gerarduzzi T, et al. Prevalence of celiac disease in at-risk and not-at-risk groups in the United States: a large multicenter study. *Arch Intern Med* 2003; **163**: 286–92.
11. Torres MI, Lopez Casado MA, Rios A. New aspects in celiac disease. *World J Gastroenterol* 2007; **13**: 1156–61.
12. Schuppan D. Current concepts of celiac disease pathogenesis. *Gastroenterology* 2000; **119**: 234–42.
13. Kutlu T, Brousse N, Rambaud C, Le Deist F, Schmitz J, Cerf-Bensussan N. Numbers of T cell receptor (TCR) alpha beta + but not of TcR gamma delta + intraepithelial lymphocytes correlate with the grade of villous atrophy in coeliac patients on a long term normal diet. *Gut* 1993; **34**: 208–14.
14. American Gastroenterological Association medical position statement: Celiac Sprue. *Gastroenterology* 2001; **120**: 1522–5.
15. Oberhuber G, Granditsch G, Vogelsang H. The histopathology of coeliac disease: time for a standardized report scheme for pathologists. *Eur J Gastroenterol Hepatol* 1999; **11**: 1185–94.
16. Trier JS. Celiac sprue. *N Engl J Med* 1991; **325**: 1709–19.
17. Brandtzaeg P, Farstad IN, Helgeland L. Phenotypes of T cells in the gut. *Chem Immunol* 1998; **71**: 1–26.
18. Marsh MN. *Gastrointestinal and oesophageal pathology*. Edinburgh: Churchill Livingstone, 1989; 161–86.
19. Alsaigh N, Odze R, Goldman H, Antonioli D, Ott MJ, Leichtner A. Gastric and esophageal intraepithelial

40

lymphocytes in pediatric celiac disease. *Am J Surg Pathol* 1996; **20**: 865–70.

20. Hayat M, Arora DS, Wyatt JI, O'Mahony S, Dixon MF. The pattern of involvement of the gastric mucosa in lymphocytic gastritis is predictive of the presence of duodenal pathology. *J Clin Pathol* 1999; **52**: 815–9.

21. Rubin CE, Brandborg LL, Flick AL, Phelps P, Parmentier C, Van Niel S. Studies of celiac sprue. III. The effect of repeated wheat instillation into the proximal ileum of patients on a gluten free diet. *Gastroenterology* 1962; **43**: 621–41.

22. Dobbins WO III, Rubin CE. Studies of the rectal mucosa in celiac sprue. *Gastroenterology* 1964; **47**: 471–9.

23. Cellier C, Cervoni JP, Patey N, et al. Gluten-free diet induces regression of T-cell activation in the rectal mucosa of patients with celiac disease. *Am J Gastroenterol* 1998; **93**: 1527–30.

24. Austin LL, Dobbins WO. Studies of the rectal mucosa in coeliac sprue: the intraepithelial lymphocyte. *Gut* 1988; **29**: 200–5.

25. Ensari A, Marsh MN, Loft DE, Morgan S, Moriarty K. Morphometric analysis of intestinal mucosa. V. Quantitative histological and immunocytochemical studies of rectal mucosae in gluten sensitivity. *Gut* 1993; **34**: 1225–9.

26. Arranz E, Bode J, Kingstone K, Ferguson A. Intestinal antibody pattern of coeliac disease: association with gamma/delta T cell receptor expression by intraepithelial lymphocytes, and other indices of potential coeliac disease. *Gut* 1994; **35**: 476–82.

27. Loft DE, Marsh MN, Crowe PT. Rectal gluten challenge and diagnosis of coeliac disease. *Lancet* 1990; **335**: 1293–5.

28. Salazar de Sousa J, Magalhaes Ramalho P, Soares J, Rodrigues C, Costa MV, da Silva A. Reaction of rectal mucosa of celiac patients to direct contact with gluten. *J Pediatr Gastroenterol Nutr* 1988; **7**: 403–5.

29. Ensari A, Ager A, Marsh MN, Morgan S, Moriarty KJ. Time-course of adhesion molecule expression in rectal mucosa of gluten-sensitive subjects after gluten challenge. *Clin Exp Immunol* 1993; **92**: 303–7.

30. Ensari A, Marsh MN, Morgan S, et al. Diagnosing coeliac disease by rectal gluten challenge: a prospective study based on immunopathology, computerized image analysis and logistic regression analysis. *Clin Sci (Lond)* 2001; **101**: 199–207.

31. Troncone R, Greco L, Mayer M, et al. In siblings of celiac children, rectal gluten challenge reveals gluten sensitization not restricted to celiac HLA. *Gastroenterology* 1996; **111**: 318–24.

32. Iovino P, Ciacci C, Sabbatini F, Acioli DM, D'Argenio G, Mazzacca G. Esophageal impairment in adult celiac disease with steatorrhea. *Am J Gastroenterol* 1998; **93**: 1243–9.

33. Aine L, Maki M, Collin P, Keyrilainen O. Dental enamel defects in celiac disease. *J Oral Pathol Med* 1990; **19**: 241–5.

34. Majorana A, Sapelli PL, Malagoli A, et al. Celiac disease and recurrent aphthous stomatitis. The clinical and immunogenetic aspects. *Minerva Stomatol* 1992; **41**: 33–40.

35. Petrecca S, Giammaria G, Giammaria AF. Oral cavity changes in the child with celiac disease. *Minerva Stomatol* 1994; **43**: 137–40.

36. Iltanen S, Collin P, Korpela M, et al. Celiac disease and markers of celiac disease latency in patients with primary Sjogren's syndrome. *Am J Gastroenterol* 1999; **94**: 1042–6.

37. Scully C, Porter SR, Eveson JW. Oral lichen planus and coeliac disease. *Lancet* 1993; **341**: 1660.

38. Bucci P, Carile F, Sangiananntoni A, D'Angio F, Santarelli A, Lo Muzio L. Oral aphthous ulcers and dental enamel defects in children with coeliac disease. *Acta Paediatr* 2006; **95**: 203–7.

39. Wierink CD, van Diermen DE, Aartman IH, Heymans HS. Dental enamel defects in children with coeliac disease. *Int J Paediatr Dent* 2007; **17**: 163–8.

40. Procaccini M, Campisi G, Bufo P, et al. Lack of association between celiac disease and dental enamel hypoplasia in a case–control study from an Italian central region. *Head Face Med* 2007; **3**: 25.

41. Lahteenoja H, Toivanen A, Viander M, et al. Oral mucosal changes in coeliac patients on a gluten-free diet. *Eur J Oral Sci* 1998; **106**: 899–906.

42. Lahteenoja H, Maki M, Viander M, et al. Local challenge on oral mucosa with an alpha-gliadin related synthetic peptide in patients with celiac disease. *Am J Gastroenterol* 2000; **95**: 2880–7.

43. Lahteenoja H, Maki M, Viander M, Toivanen A, Syrjanen S. Local challenge of oral mucosa with gliadin in patients with coeliac disease. *Clin Exp Immunol* 2000; **120**: 38–45.

44. Ellis HJ, Ciclitira PJ. The mouth – an accessible region for gluten challenge. *Clin Exp Immunol* 2000; **120**: 10–1.

45. Carroccio A, Campisi G, Iacono G, et al. Oral mucosa of coeliac disease patients produces antiendomysial and antitransglutaminase antibodies: the diagnostic usefulness of an in vitro culture system. *Aliment Pharmacol Ther* 2007; **25**: 1471–7.

46. Revised criteria for diagnosis of coeliac disease. Report of Working Group of European Society of Paediatric Gastroenterology and Nutrition. *Arch Dis Child* 1990; **65**: 909–11.

47. Petras RE. Nonneoplastic intestinal diseases. In: *Sternberg's diagnostic surgical pathology, 4th ed.* Philadelphia: Lippincott Williams & Wilkins, 2004; 1475–541.

48. Rosai J. *Rosai and Ackerman's Surgical Pathology, 9th ed.* New York: Mosby, 2004; 716.

49. Drut R, Rua EC. The histopathology of pediatric celiac disease: order must prevail out of chaos. *Int J Surg Pathol* 2001; **9**: 261–4.

50. Sedghizadeh PP, Shuler CF, Allen CM, Beck FM, Kalmar JR. Celiac disease and recurrent aphthous stomatitis: a report and review of the literature. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2002; **94**: 474–8.

51. Aine L. Dental enamel defects and dental maturity in children and adolescents with coeliac disease. *Proc Finn Dent Soc* 1986; **82** (Suppl. 3): 1–71.

52. Rasmusson CG, Eriksson MA. Celiac disease and mineralisation disturbances of permanent teeth. *Int J Paediatr Dent* 2001; **11**: 179–83.

53. Bossu M, Bartoli A, Orsini G, Luppino E, Polimeni A. Enamel hypoplasia in coeliac children: a potential clinical marker of early diagnosis. *Eur J Paediatr Dent* 2007; **8**: 31–7.

54. Prati C, Santopadre A, Baroni C. Delayed eruption, enamel hypoplasia and caries in childhood celiac disease. *Minerva Stomatol* 1987; **36**: 749–52.

55. Lahteenoja H, Toivanen A, Viander M, et al. Increase in T-cell subsets of oral mucosa: a late immune response in patients with treated coeliac disease?. *Scand J Immunol* 2000; **52**: 602–8.

56. Squier CA, Kremer MJ. Biology of oral mucosa and esophagus. *J Natl Cancer Inst Monogr* 2001; **29**: 7–15.

57. Brandtzaeg P, Farstad IN, Haraldsen G. Regional specialization in the mucosal immune system: primed cells do not always home along the same track. *Immunol Today* 1999; **20**: 267–77.

58. Maki M, Collin P. Coeliac disease. *Lancet* 1997; **349**: 1755–9.
59. Stenn KS, Balin AK, Higgins T, Stenn JO. Spongiosis. *J Am Acad Dermatol* 1981; **5**: 213–4.
60. Weedon D. The spongiotic reaction pattern. In: *Skin Pathology, 2nd ed.* New York: Churchill Livingstone, 2002; 97–119.
61. Brodersen I, Frentz G, Thomsen K. Eosinophilic spongiosis in early pemphigus foliaceus. *Acta Derm Venereol* 1978; **58**: 368–9.
62. Isogai R, Kawada A, Aragane Y, Amagai M, Tezuka T. A case of herpetiform pemphigus with anti-desmoglein 3 IgG autoantibodies. *J Dermatol* 2004; **31**: 407–10.
63. Brod C, Fierlbeck G, Metzler G, Sonnichsen K, Rocken M, Schaller M. Desmoglein 1-negative, desmoglein 3-positive pemphigus herpetiformis with involvement of oral mucous membranes. *J Dtsch Dermatol Ges* 2005; **3**: 280–2.
64. Bayer-Garner I, Dilday B, Sanderson R, Smoller B. Acantholysis and spongiosis are associated with loss of syndecan-1 expression. *J Cutan Pathol* 2001; **28**: 135–9.
65. Contreras E, Carlos R. Oral melanoacanthosis (melanoacanthoma): report of a case and review of the literature. *Med Oral Patol Oral Cir Bucal* 2005; **10**: 11–2.
66. Migliari DA, Penha SS, Marques MM, Matthews RW. Considerations on the diagnosis of oral psoriasis: a case report. *Med Oral* 2004; **9**: 300–3.
67. De Rossi SS, Greenberg MS. Intraoral contact allergy: a literature review and case reports. *J Am Dent Assoc* 1998; **129**: 1435–41.
68. Neville BW, Damm DD, Allen CM, Bouquot JE. Allergies and immunologic diseases. In: *Oral and maxillofacial pathology, 2nd ed.* Philadelphia: W. B. Saunders Co., 2002; 285–90.
69. Jurge S, Kuffer R, Scully C, Porter SR. Mucosal disease series. Number VI. Recurrent aphthous stomatitis. *Oral Dis* 2006; **12**: 1–21.
70. Campisi G, Di Liberto C, Carroccio A, et al. Celiac disease: oral ulcer prevalence, assessment of risk and association with gluten-free diet in children. *Dig Liver Dis* 2008; **40**: 104–7.
71. Demarosi F, Lodi G, Carrassi A, Moneghini L, Sarina B, Sardella A. Clinical and histopathological features of the oral mucosa in allogenic haematopoietic stem cell transplantation patients. *Exp Oncol* 2007; **29**: 304–8.

Acknowledgments