Ultrastructural Aspects of Mucosas in Endemic Pemphigus Foliaceus

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Objective: To investigate whether ultrastructural changes present in clinically normal oral mucosa could occur in the mucosas of patients with endemic pemphigus foliaceus (EPF) or fogo selvagem (wildfire).

Patients: Surgical biopsy specimens were taken from the foreskin of 8 patients with EPF and 3 control subjects, the uterine cervix and vaginal wall of 9 patients with EPF and 2 controls, and the oral mucosa of 5 patients with EPF and 4 controls. The patients received a clinical and histopathologic diagnosis of EPF and all had clinically normal oral and genital mucosas.

Results: In the patients with EPF, widening of the intercellular spaces and distended, elongated cytoplasmic projections, the tips of which contained desmosomes and were sometimes disassembled, were evident in all 4 regions studied. At the periphery of the spinous cells, cytoplasmic vesicles apparently containing intact or fragments of desmosomes or half-desmosomes were seen.

Conclusions: The ultrastructural lesions found in the mucosas studied are similar to those previously described in the literature for the oral mucosa of patients with EPF. In the cases of EPF, even though the desmosomal changes occurred in all epithelial layers, blisters did not occur in the mucosas by possible coexpression of desmoglein 1 and desmoglein 3.

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Endemic pemphigus foliaceus (EPF) or fogo selvagem (wildfire) is an autoimmune blistering disease considered a variant of pemphigus foliaceus. Its occurrence is frequent in deforested areas in Brazil, representing a serious public health problem.

Since the antiepithelial antibodies were first demonstrated, significant progress has been made in the study of pemphigus. However, many obscure points remain, such as the acantholytic phenomena in the pathogenesis of the disease, especially the absence of lesions in the oral mucosa of patients with pemphigus foliaceus.

Perry1 and Azulay2 reported involvement of the mucosas in EPF, particularly the oral mucosa, as being rarely observed and unsupported by convincing laboratory evidence. Mucosal lesions, even in the more serious clinical forms, do not occur in this disease. Using direct immunofluorescence, Takahashi3 demonstrated the surprising presence of pemphigus foliaceus autoantibodies in the intercellular spaces of the epithelium of the oral and esophageal mucosas of most patients. Rivitti et al4 observed the predominance of IgG4 in both the oral mucosa and the epidermis. Positive direct immunofluorescence on the epithelium of the oral mucosa shows no correlation with either the titers of EPF autoantibodies in the patient’s serum or the clinical forms of EPF. These findings are in opposition to the well-established correlation between the titers of EPF autoantibodies in the patient’s serum and the severity of the skin disease.

Marcucci et al5 and Marcucci6 described the ultrastructure of the jugal mucosa of patients with EPF compared with that of a control group. They found changes in the oral mucosa that could not be described as characteristic of EPF. There was widening of the intercellular spaces, with dissolution of the intercellular substance, lamellar bodies were frequently present, and the desmosomal junctions were irregularly distributed.

Sotto et al7, who studied skin and oral biopsy specimens, suggested that the initial lesion in pemphigus acantholysis involves the glycocalyx and might be caused by interaction with intercellular antibodies present in the patient’s serum.

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PATIENTS AND METHODS

The patients studied were outpatients who presented to the Dermatology Service of the Federal University of Minas Gerais, Belo Horizonte, Brazil, and all had active EPF lesions. The diagnosis was based on clinical and histopathologic data (Table). The oral and genital mucosas of all patients were clinically examined and no clinical lesions were found. The cases were classified according to the criteria of the Cooperative Research Group for the Study of Fogo Selvagem as described by Diaz et al: (1) localized form (forme fruste); (2) generalized forms (bullous-exfoliative, exfoliative-erythrodemic, and disseminated keratotic plaques); and (3) hyperpigmented forms. None of the patients with EPF was receiving treatment when examined or at the time samples were collected.

Surgical biopsy specimens were taken from the internal face of the foreskin of 8 patients with EPF and 3 control subjects, the uterine cervix and vaginal wall of 9 patients with EPF and 2 controls, and the oral mucosa of 3 patients with EPF (3 men and 2 women) and 4 controls (Figures 1, 2, 3, and 4).

The examinations and their purpose were described to all patients and appropriate consent was obtained in writing. The research project was approved by the Ethics Committees of Hospital das Clínicas and the Clinical Practice Department of the School of Medicine, Federal University of Minas Gerais.

The samples for electron microscopy were fixed for 3 hours in 3% glutaraldehyde in phosphate-buffered saline (0.1M, pH 7.2), postfixed for 2 hours in 1% osmium tetroxide in the same buffer, and contrasted en bloc for 30 minutes in distilled water containing 0.3% tannic acid. Dehydration was done with an ascending series of alcohol, followed by infiltration in a mixture of acetone–Epon 812–Araldite 502 and Epon 812–Araldite 502 (Epon 812 and Araldite 502; Polysciences, Inc, Warrington, Pa) and embedding in the same resin at 60°C. Semithin sections stained with toluidine blue were used for orientation of the samples and identification of the areas to be isolated for ultrastructural examination. Ultrathin sections stained with uranyl acetate and lead citrate were examined and photographed with an electron microscope (EM9 52; Carl Zeiss, Gottingen, Germany).

Hietanen and Lounatmaa,8 while studying the oral mucosa of healthy individuals and patients with pemphigus vulgaris or pemphigus erythematosus, observed widening of the intercellular spaces and a smaller than normal number of desmosomes in both the involved and the unaffected mucosa. Hietanen et al9 examined the unaffected oral mucosa of 7 patients with pemphigus (including 3 with pemphigus erythematosus and 1 with pemphigus foliaceus); in 5 of the 7 patients, they found widening of the intercellular spaces compared with controls.

Roscoe et al10 reproduced the EPF lesions in neonatal BALB/c mice and confirmed the pathogenicity of the antiepithelial autoantibodies in EPF. In the beginning, binding of the autoantibodies in the intercellular spaces of the epidermis was observed, followed by widening of such spaces between the desmosomes and desmosomal disruption.

Futamura et al11 studied the dynamics of the ultrastructural acantholytic changes induced by EPF IgG in rats. Electron microscopy showed diffuse IgG binding on the surface of the keratinocytes. Swelling of the intercellular spaces in the basal layer was observable by immunoelectron microscopy as early as 1 hour after intraperitoneal injection of IgG in neonatal BALB/c mice. At 12 hours, microvillous formations with intact desmosomes at the tip of the projections were present. Desmosomes split into halves, forming half-desmosomes, and primary acantholysis that affected the granular layer was clearly evident between 12 and 24 hours. The attachment plaques of the half-desmosomes gradually disappeared and the tonofilaments retracted into the cytoplasm. The detached keratinocytes showed cytoplasmic vacuolization, swollen mitochondria, and internalization of desmosomes and half-desmosomes. Desmosomal adhesion was impaired in all layers of the epidermis, even though blisters occurred only in the more differentiated cell areas.

In pemphigus foliaceus, patients develop blisters within the granular layers of the superficial epidermis.

<table>
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<tr>
<th>Surgical Biopsy Site</th>
<th>Clinical Findings*</th>
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<th>Skin Histopathologic Findings</th>
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<td>5 Positive, 3 negative</td>
<td>8 Positive</td>
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<td>3 Normal skin and mucosas</td>
<td>3 Normal</td>
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<tr>
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<td>6 Positive, 3 negative</td>
<td>9 Positive</td>
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<td>2 Normal skin and mucosas</td>
<td>2 Normal</td>
<td>2 Negative</td>
</tr>
<tr>
<td>Oral mucosa (n = 7)</td>
<td>4 Generalized, 1 localized</td>
<td>4 Positive, 1 negative</td>
<td>5 Positive</td>
<td>5 Superficial acantholytic blisters</td>
<td>2 Normal skin and mucosas</td>
<td>2 Normal</td>
<td>2 Negative</td>
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*According to the criteria of the Cooperative Research Group for the Study of Fogo Selvagem as described by Diaz et al.15
but not in the mucous membrane. Shirakata et al explained the absence of blisters in squamous mucosal epithelia in pemphigus foliaceus by much lower expression of desmoglein 1 (Dsg1) than Dsg3. The blocking of...
Dsg1 by autoantibodies would be compensated for Dsg3 production within the same desmosomes and the cell-cell adhesion in the mucosas would be maintained. Mahoney et al\textsuperscript{13} demonstrated that mucous membranes coexpress Dsg1 and Dsg3 throughout all cell layers and either Dsg1 or Dsg3 alone is sufficient to maintain cell-cell adhesion. Therefore, the autoantibodies directed against Dsg1 in pemphigus foliaceus are inefficient at causing blisters. Wu et al\textsuperscript{14} found that the distribution of Dsg1 is similar in the skin of neonates and adults; however, Dsg3 is present on the surface of keratinocytes throughout the epidermis in neonatal skin, whereas in adult skin it is present only in the deep epidermis. Consequently, they hypothesized that the expression of Dsg3 in the superficial epidermis provides protection against the formation of blisters induced by pemphigus foliaceus.

Considering all of these electron microscopy studies, the sequence of events that occurred in the epidermis of the patients with pemphigus is demonstrated. First, antibody binding would occur in the intercellular spaces, followed by swelling and widening of the intercellular spaces and the formation of cytoplasmic projections, with desmosomes remaining intact at the tip of the projections. Finally, there would be rupture and internalization of desmosomes. The purpose of this article is to determine, by electron microscopy, whether early changes preceding the phenomenon of acantholysis, as described in the literature in the oral mucosa, are detectable in the mucosas of the uterine cervix, vaginal wall, and internal surface of the foreskin. In the cases of EPF, even though the desmosomal changes occurred in all epithelial layers, blisters did not develop in mucosas by possible coexpression of Dsg1 and Dsg3.

RESULTS

The patients received a clinical and histopathologic diagnosis of EPF and all had clinically normal oral and genital mucosas (Table).

In the patients with EPF, epithelial lesions in the 4 regions studied (oral mucosa, internal surface of the foreskin, uterine cervix, and vaginal wall) occurred throughout epithelial layers and were more severe in the spinous one. Such lesions were characterized by widening of the intercellular spaces and distended, elongated cytoplasmic projections (Figures 1A and C, 2A, 3A, and 4A), the tips of which contained desmosomes, sometimes disassembled. At the periphery of the spinous cells, cytoplasmic vesicles containing intact or fragmented desmosomes or half-desmosomes were seen (Figure 1C).
The intercellular spaces of the control mucosas were not widened and the cells exhibited no cytoplasmic vesicles (Figures 1B and D, 2B, 3B, and 4B). Of all mucosas, that of the foreskin showed the largest number of bundles of intermediate filaments, in addition to more elongated and more numerous desmosomes and half-desmosomes (Figure 4B).

**COMMENT**

Since the works of Wilgram et al, who, studying pemphigus vulgaris, postulated that the primary lesion occurs in the intercellular space, with changes in the desmosomes, few incursions have been made into electron microscopy on the various forms of pemphigus. Most of the initial studies agreed that the intercellular space was the chief site of involvement, whether the lesion was located in the intercellular cement, the spinous cell membranes, the glycocalyx, or, finally, the desmosomes. Similar findings to these were reported by Barros, Konrad et al, and Sotto et al in EPF, who found dissolution of the intercellular cement and widening of the intercellular spaces, leading to irregular distribution of the desmosomal junctions. The oral mucosa in EPF was studied by Marcucci, Sotto et al, and Akiyama et al, using electron microscopy. The surprising findings of these authors were similar to those relating to the skin of the patients (ie, widening of the intercellular spaces and irregularly distributed desmosomes, without correspondence, however, to the clinical forms of the disease).

Based on such findings, we tried to determine, in the present study, whether the lesions described by Marcucci, Sotto et al, and Akiyama et al occur in mucosas other than the oral mucosa. Thus, we have demonstrated for the first time, to our knowledge, that widening of the intercellular spaces; elongated, distended cytoplasmic projections; and desmosomal disassembly occur also in the epithelium of the mucosa of the uterine cervix, vaginal wall, and internal surface of the foreskin. Similar to what is observable in the oral mucosa, changes are present that would precede the appearance of acantholysis. Yet, because acantholysis is not completed, these mucosas are clinically normal in EPF.

The finding of cytoplasmic vesicles corroborates the observation of Futamura et al, who reproduced this event in experimental animals. Internalization of desmosomes in cytoplasmic vesicles in pemphigus vulgaris was reported by Iwatsuki et al. In fresh skin specimens rapidly processed for electron microscopy, these authors observed endocytosis of split desmosomes, concluding that the antigen-antibody complexes are internalized by means of endocytosis.

Acantholysis is a process that takes place in successive stages and demands time for its development. Such hypothesis is confirmed by Hu et al and Futamura et al in experimental studies with cultured normal human skin and neonatal BALB/c mice, respectively. Twelve hours after the experiment was initiated, widening of the intercellular spaces and microvillous formation were already observable. At 12 to 24 hours, split desmosomes were present. At approximately 72 hours, isolated cells without desmosomes were found.

This sequential process is questionable in areas of accelerated cell renewal, such as the mucosas, in which a longer period would be required for acantholysis to occur. On the other hand, as was demonstrated by Akiyama et al, the possibility exists that a different antigenic molecule or a different epitope from the same molecule might be recognized by the autoantibodies of EPF, causing various clinical manifestations.

In this transmission electron microscopy study, confirmation was obtained that the other analyzed mucosas, even if clinically normal, show similar changes to those observed in the oral mucosa in EPF (ie, widening of the intercellular spaces and digitation and loss of adhesion in the desmosomal areas). Thus, an unequivocal explanation for the absence of mucosal lesions in EPF remains to be found.

According to Shirakata et al and Mahoney et al, there is different distribution of Dsg1 and Dsg3 between skin and mucosas in pemphigus foliaceus and either Dsg1 or Dsg3 alone is sufficient to maintain the cell-cell adhesion. For the EPF, no blisters are apparent in the mucosas, although the anti-Dsg1 antibodies binding the Dsg3 high production, not blocked, would maintain cell-cell adhesion. The ultrastructural epithelial...
changes seen are probably caused by the action of the anti-Dsg1 antibodies.

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REFERENCES


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