

# An Electron Microscopic Study of Oral Lesions in Erythema Multiforme

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**The ultrastructure of oral lesions in 6 patients with erythema multiforme (EM) and of one apparently clinically healthy oral mucosa in one patient with recurrent EM during remission is described. Alterations were observed in epithelium, basal lamina and lamina propria. Both intercellular and intracellular oedema, intracellular vacuolization, decreased numbers of desmosomes, and also loss of cytoplasmic organelles and occasional nuclei were noted in the epithelium. Inflammatory cells – mainly lymphocytes – were found intra-epithelially. Several discontinuities together with some evidence of duplication of the basal lamina were seen in five of the six lesional mucosa specimens. The inflammatory infiltrate in the lamina propria consisted mainly of lymphocytes, although plasma cells, neutrophilic and eosinophilic leukocytes, macrophages and mast cells were also found. Some of the mast cells were partly degranulated. The apparently clinically healthy oral mucosa in the patient with EM in remission showed mild inflammatory changes. The changes observed in the lesional mucosa in EM are thus according to our study mostly non-specific inflammatory alterations and are not pathognomonic for EM. Key words: Oral mucosa; Ultrastructure; Mast cells.**

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To our knowledge, only two electron microscopic studies of oral lesions in erythema multiforme (EM) have been published (1, 2). Newman (1) reported viral type bodies in filtrates of saline mouth washings from patients with EM. The ultrastructural features of the oral lesions in EM were not dealt with in that study. In an electron microscopic study of oral mucosal lesions in EM, von Bülow et al. (2) demonstrated two types of oedematous changes in the stratum spinosum of the epithelium, namely an intercellular and an intracellular oedema. In the areas of intracellular oedema the epithelial cells were vacu-

olated and their cytoplasmic organelles had disappeared. An alteration in chromatin pattern, either as a peripheral clumping or as a disintegration, with disappearance of the nuclear membrane could also be seen in the nuclei of the epithelial cells. The same authors reported that the cells in the areas of intercellular oedema demonstrated all the cytoplasmic organelles and had apparently normal nuclei. Lymphocytes were noted between the epithelial cells in the spinal and basal cell layers. The basement membrane was unbroken and no changes were seen in the connective tissue. Nor were there any signs of viral infection.

The aim of the present study was to report on the ultrastructure of oral lesions in EM.

## PATIENTS AND METHODS

Biopsy specimens from 7 patients with EM were examined in this study. Six biopsy specimens were obtained from oral lesions from the same number of patients at the onset of their EM episode. One biopsy specimen was taken from a patient with recurrent EM from an apparently clinically healthy oral mucosa during remission (patient 3) (Table 1). A control specimen for transmission electron microscopy (TEM) was obtained during a wisdom tooth operation from the apparently clinically healthy oral buccal mucosa of a healthy volunteer (female, 26 yrs) with no history of EM or other mucosal disease. Informed consent was obtained from all the patients and the study was approved by the Medical Ethics Committee of the Department of Dermatology, Helsinki University Central Hospital.

The clinical and histological criteria for the diagnosis of EM were those proposed by Huff et al. (3). All patients fulfilled these criteria. Four patients had previously had a herpes simplex virus (HSV) infection which could have been a triggering factor, while the remaining 3 had EM of unknown etiology. The mean age of the patients was 32.4 years. Patients 1 and 4 were receiving systemic corticosteroid medication (prednisone 30 mg and 40 mg a day, respectively) at the time of the biopsy. None of the other patients was receiving any systemic medication, and local treatment of the oral mucosa was started after biopsy. Citanest-Octapressin® (30 mg/ml prilocain + 0.54 µg/ml felypressin) (Astra, Södertälje, Sweden) was used for local infiltration anesthesia adjacent to the lesional mucosa.

Table I. Information on the patients with erythema multiforme

Patient	Sex	Age	Lesion	Site of biopsy	Previous triggering factor
1. M.L.	M	18	Macula	Lip mucosa	HSV
2. A.S.	M	25	Erosion	Buccal mucosa	Unknown
3. A.L. <sup>a</sup>	M	25	-	Buccal mucosa	Unknown
4. J.K.	M	29	Erosion	Buccal mucosa	Unknown
5. H.L.	M	39	Erosion	Buccal mucosa	HSV
6. P.L.	F	15	Erosion	Buccal mucosa	HSV
7. E.U.	F	76	Macula	Palatal mucosa	HSV

Mean age = 32.4 years.

<sup>a</sup>Apparently clinically healthy oral mucosa from a patient with erythema multiforme in remission.

The biopsy specimens were immediately cut into halves, one of which was fixed in 10% formalin and prepared for light microscopy. The other half was cut into pieces measuring approximately 1×1×2 mm and prepared for TEM.

Immediately after removal, the biopsy specimen was prefixed in cold (+4°C) phosphate-buffered glutaraldehyde (1.5%) for 1 hour, washed with sodium phosphate buffer (0.1 M; pH 7.4) and postfixed in buffered 1% osmium tetroxide for 90 min. After postfixation the specimens were washed again with sodium phosphate buffer (0.1 M; pH 7.4), dehydrated in a graded ethanol series and treated with propylene oxide for 30 min. The specimens were then treated with a mixture containing equal parts of propylene oxide and Epon LX 112 for 2–3 h and then embedded in Epon LX 112. Ultrathin sections were cut with an ultramicrotome (LKB, ultramicrotome system, 2188 Ultratome®). The specimens were polymerized at 40°C for 20 h and then at 60°C for 48 h, stained with uranyl acetate and

lead citrate (LKB 2168 Ultrastainer Carlsberg system) and examined with a JEOL CX-1200 transmission electron microscope operating at 60 kV at the Department of Electron Microscopy, University of Helsinki. Some four to six sections from each specimen were viewed in the microscope.

## RESULTS

Alterations were observed in the oral epithelium, basal lamina and lamina propria.



Fig. 1. Oral lesional mucosa from a patient with EM (patient 6). The number of desmosomes is decreased and the intercellular spaces are widened. Two inflammatory cells (*I*, *i*) are seen intra-epithelially. Discontinuities (*small arrows*) can be seen in the basal lamina. A plasma cell (*large arrowhead*) can be seen in the lamina propria. *e*=epithelium, *ct*=connective tissue ×9000. Bar 1 µm.

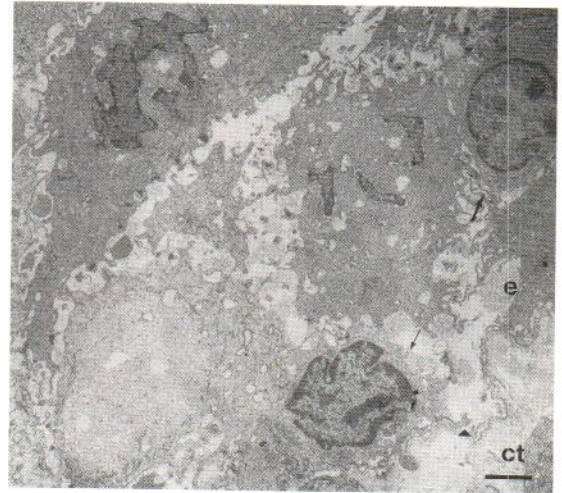


Fig. 2. Oral lesional mucosa from a patient with EM (patient 4). Some of the basal epithelial cells have been destroyed and have lost their cytoplasmic organelles as well as their nucleus. A discontinuity (*arrowhead*) can be seen in the basal lamina. A lymphocyte (*small arrow*) and a plasma cell (*large arrow*) are seen intra-epithelially. *e*=epithelium, *ct*=connective tissue ×5000. Bar 2 µm.

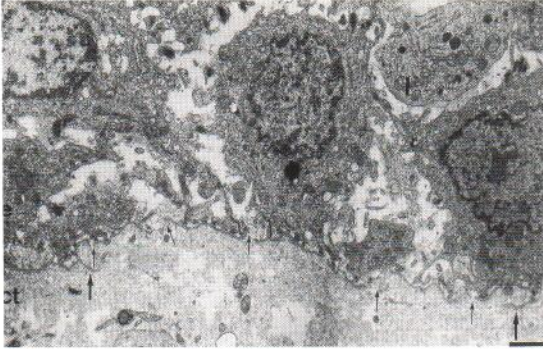


Fig. 3. Several discontinuities (*small arrows*) and also evidence of duplication (*large arrows*) can be seen in the basal lamina from oral lesion of a patient with EM (patient 5). The basal cell layer shows decreased numbers of desmosomes and widened intercellular spaces. An inflammatory cell can be seen intra-epithelially (*I*). *e* = epithelium, *ct* = connective tissue.  $\times 9000$ . Bar  $1 \mu\text{m}$ .

#### Ultrastructure of control specimen

The apparently clinically healthy oral buccal mucosa of a healthy volunteer with no history of EM or other mucosal disease did not display any pathological changes ultrastructurally.

#### Ultrastructure of apparently clinically healthy oral mucosa from patient with EM in remission

The specimen from patient no. 3 showed changes in the basal cell layer. These were a widening of the intercellular space, intercellular oedema, and a reduction in the number of desmosomes. The inflammatory infiltrate in both the epithelium and upper

lamina propria consisted of lymphocytes, mast cells and plasma cells.

#### Ultrastructure of epithelium of lesional mucosa

Most superficial parts of the epithelium had a normal appearance. In the upper stratum spinosum, intercellular oedema was noticed in five of the six specimens and widening of the intercellular space in three of the six. Both intercellular and intracellular oedema with vacuolization, loss of cytoplasmic organelles and destruction of oral epithelial cells were found in the upper stratum spinosum of three specimens.

The oedematous changes increased towards the lower spinous and basal cell layers, where both intercellular and intracellular oedema were observed in five of the six specimens. The numbers of desmosomes and hemidesmosomes were decreased in all the specimens, and stretching of desmosomal areas and/or loss of their architecture was noticed in four of the six specimens. In the most damaged areas of the basal cell layer the cells were vacuolated and had lost all their cytoplasmic organelles and occasionally their nucleus (Figs. 1, 2).

The nuclear membrane had disappeared in some basal cells and a disintegration or accumulation of chromatin to the periphery of nucleus was seen. In the areas of slight intracellular oedema the basal cells presented a pyknotic nucleus, some mitochondria and glycogen. Thick bundles of tonofilaments were occasionally seen bulging towards the desmosomes in two of the six specimens. Lengthening of cell projections of adjacent cells was noted also in

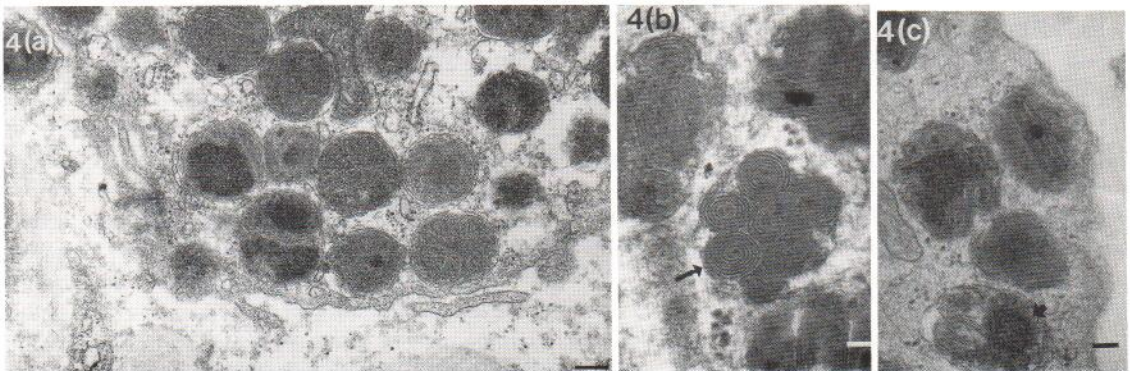


Fig. 4a,b,c. Mast cell granules from the lesional mucosa of a patient with EM (*a*=patient 4) and from apparently clinically healthy oral mucosa in the patient with EM in remission (*b* and *c*=patient 3). Mast cell granules had a characteristic substructure composed of coarsely interlacing strands, scrolls (*small arrows*) or particles (*large arrows*) mostly in combination, but occasionally also alone in the same cell.  $\times 40000$ , bar  $200 \text{ nm}$  (*a*);  $\times 100000$ , bar  $50 \text{ nm}$  (*b*); and  $\times 60000$ , bar  $100 \text{ nm}$  (*c*).

two of the six specimens in areas of intercellular oedema.

Inflammatory cells – mainly small lymphocytes – but also neutrophilic and/or eosinophilic leukocytes and plasma cells were found intra-epithelially (Figs. 1, 2).

#### *Ultrastructure of basal lamina of lesional mucosa*

Several discontinuities and/or thinning of the basal lamina were seen in five of the six specimens, whereas one had an apparently normal-looking basal lamina. Some evidence of duplication in the form of additional short strands of basal lamina was noted in five of the six specimens (Fig. 3).

#### *Ultrastructure of upper lamina propria of lesional mucosa*

Inflammatory infiltrate was seen in the upper lamina propria of all specimens. Most of the inflammatory mononuclear cells found were lymphocytes. Macrophages and plasma cells were also seen. Mast cells were seen in four of the six specimens near the basal lamina or in the upper lamina propria. Their cytoplasm was filled with granules with a substructure composed of coarsely interlacing strands, scrolls and/or lamellar inclusions (Fig. 4a,b,c). Some of the mast cells were partly degranulated. Only a few neutrophilic leukocytes were seen in two of the six specimens and eosinophilic leukocytes in one of the six in the lamina propria.

Perivascular inflammation was seen in four of the six specimens. The perivascular infiltrate contained mainly lymphocytes.

No changes in collagen fibres were found. Intercellular oedema was visible in five of the six specimens. The vascular basal lamina of the vessels showed thinning in only two specimens and thickening in one.

No difference was found between the biopsy specimens from patients 1 and 4, both of whom were receiving systemic corticosteroid treatment at the time of the biopsy, and the specimens from patients undergoing no treatment.

## DISCUSSION

Orfanos et al. (4) believe that two ultrastructural types of EM occur in skin: dermal and epidermal. They reported that in the dermal type of EM the

changes within the epidermis are mild, consisting of intercellular and intracellular oedema, vacuolar degeneration, dyskeratosis and loss of desmosomes. In connective tissue, however, they reported swelling of endothelial cells, deposits of fibrinoid material throughout the collagen and around the capillaries, and an irregular arrangement of disoriented collagen fibrils. In the epidermal type of EM they observed extensive damage to the dermo-epidermal junction and the lower Malpighian cells, together with numerous interruptions of the basal lamina.

In the present study, most of the specimens taken from oral lesions of EM showed alterations in the epithelium, basal lamina and lamina propria and could not be classified into either of the above two categories; instead, they were more a mixed dermal-epidermal type of EM. This is in agreement with Ackerman & Ragaz (5), who stated that EM in the skin invariably involves the connective tissue and epidermis simultaneously. The changes observed depend on the stage of the lesion.

Caulfield & Wilgram (6) and Putkin & Fellner (7) found both varying degrees of intracellular and intercellular oedema and reduced numbers of desmosomes as well as loss of their architecture in some epithelial cells in EM skin lesions. These findings accord with ours concerning the ultrastructure of the epithelium in oral mucosal lesions of EM.

In the study by von Bülow et al. (2) on oral EM lesions, the basal lamina was found to be intact. We found several discontinuities of the basal lamina in five of the six lesional mucosa specimens. Duplication of the basal lamina was also noted, possibly indicating areas of healing. Similar findings have been reported by Caulfield and Wilgram (6) in skin lesions of EM. Orfanos et al. (4) have also reported multiple interruptions with severe damage of the basal lamina in the epidermal type of EM.

An inflammatory infiltrate consisting mainly of lymphocytes but also occasional macrophages, plasma cells and mast cells was found in the upper lamina propria and perivascularly. This is in agreement with most of the previous studies (4, 6, 8). We also found lymphocytes, neutrophilic and eosinophilic leukocytes and plasma cells intra-epithelially.

In the present study, mast cells were found in the upper lamina propria and often near the basal lamina. They contained granules with a characteristic substructure, which appeared as scrolls, particles or crystals, usually in combination but also alone, as described by Weinstock & Albright (9). In our study

we identified large, fully granulated as well as partly degranulated mast cells which had released some of their granules into the connective tissue matrix. Mast cells are known to play an important role in allergic reactions as well as in inflammatory processes induced both immunologically and non-immunologically.

In our study, most of the endothelial cells of the capillaries had a normal appearance. Only two specimens showed thinning of the vascular basal lamina and one specimen occasional thickening. However, in the skin lesions of EM vascular changes in the form of endothelial cytoplasmic swelling, vacuolization, discontinuities and duplications of the vascular basal lamina and separation of the endothelial cells have been reported (4, 6, 10). According to Tonnesen et al. (10), venular damage correlates with the degree of infiltration by lymphocytes, which is the primary effector cell, and they suggest that the venule is a primary target for injury.

We found no difference in the ultrastructure of biopsy specimens between the 2 patients receiving systemic corticosteroid treatment at the time of the biopsy, and patients receiving no treatment. However, Caulfield & Wilgram (6) found layering of the basement membrane to be a prominent change in one patient being treated with corticosteroids. The biopsy specimen taken prior to corticosteroid therapy also showed some evidence of duplication, but to a lesser extent. In the present study, the corticosteroid treatment of the 2 patients was started only a few days prior to the biopsy, which could explain the difference between the present study and that by Caulfield & Wilgram (6).

We found inflammatory changes in the apparently clinically healthy oral mucosa from the patient with EM in remission. Venular alterations and slight perivascular infiltrate of lymphocytes have also been reported in uninvolved skin in a patient with recurrent EM (10).

Viral type bodies have been reported by Newman (1), who used TEM to examine filtrates of saline mouth washings from patients with EM. In our study on biopsy specimens, no signs of viral infection were found by TEM. However, this does not exclude the possibility of HSV infection. The presence of HSV antigens in keratinocytes in EM skin lesions has

been documented by Orton et al. (11) using an indirect immunofluorescence method.

In conclusion, the oral mucosal lesions of EM involve the epithelium, the basal lamina and the lamina propria. However, the changes observed are mostly non-specific inflammatory alterations and are not pathognomonic for EM.

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