

Mast Cells in Oral Erythema Multiforme

H. RUOKONEN

Departments of Oral and Maxillofacial Surgery and Oral Pathology, Institute of Dentistry, University of Helsinki, Finland

We compared the number of mast cells in erythema multiforme lesions, in clinically healthy mucosa between the EM attacks and in healthy mucosa from healthy volunteers. The mast cell count in patients with erythema multiforme was numerically higher than in healthy controls, but the differences were not statistically significant. In erythema multiforme lesions the mast cell count was low in the intensely inflamed superficial lamina propria, but high in normal appearing mucosa between the attacks suggesting local mast cell degranulation in the most intensely inflamed areas. Key word: Oral mucosa.

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H. Ruokonen, Department of Oral and Maxillofacial Surgery, Mannerheimintie 172, SF-00300 Helsinki, Finland.

Herpes simplex virus (HSV) infection and drugs are the main precipitating factors of erythema multiforme (EM) (1-3). It has been speculated that HSV-associated EM represents a hypersensitivity phenomenon induced by a recurrent HSV infection and drug-associated EM a hypersensitivity reaction to a drug (1).

Mast cells (MC) are important in hypersensitivity reactions but also in inflammatory processes in general (4, 5). Activation of MC causes a release of different mediators and production and release of mediators from other cells (5).

The aim of this study was to examine the possible involvement of mast cells in the pathogenesis of oral EM lesions. The MC counts in oral EM lesions were compared with those in non-affected mucosa between the attacks in patients and healthy volunteers. The findings were also correlated with the intensity of inflammation.

PATIENTS AND METHODS

Sixteen biopsy specimens were obtained under local anaesthesia from 4 men and 3 women (age 15-42 years) with EM and from 4 healthy subjects of same age. Seven of the specimens were obtained from EM lesions at the onset of their EM episode. With the exception of 2 patients, all the lesional specimens were taken from buccal lesion. One specimen was obtained from gingival blister and the other one from buccal maculae. Two patients had previously had an HSV infection which could have been a triggering factor, while the remaining 5 had EM of unknown aetiology. Five of the patients were suffering from recurrent EM, and biopsy specimens were also taken from healthy buccal mucosa between attacks. Four specimens were taken from healthy buccal mucosa from 4 healthy subjects to serve as control specimens. The findings were compared. Informed consent was obtained from all patients and volunteers. The patients were treated at the Department of Dermatology, Helsinki University Central Hospital, and the study was approved by the Medical Ethics Committee of the hospital.

The clinical and histological criteria used for the diagnosis of EM were those proposed by Huff et al. (1). None of the patients was receiving any systemic medication, and local treatment of the oral mucosa was started first after biopsy. Citanest-Octapressin® (30 mg/ml

prilocain + 0.54 µg/ml felypressin) (Astra, Södertälje, Sweden) was used for local infiltration anaesthesia adjacent to the lesional mucosa.

Mast cells were stained from formalin-fixed paraffin-embedded specimens with toluidine blue (TB) and with naphthol-ASD-chloroacetate (N-ASD-C).

Toluidine blue staining

The deparaffinized 5 µ specimens were treated with TB for 10 min, washed under running tap water for 10 min, dehydrated, cleared and mounted.

Naphthol-ASD-chloroacetate staining

For staining of non-specific esterase the deparaffinized 5 µ sections were incubated in 85 µl of 4% pararosaniline in 2N HCl blended with 85 µl of 4% sodium nitrate for exactly 30 s. 60 ml 0.1 M Michael's sodium barbital buffer was added slowly. The pH was adjusted to 6.3 (± 0.02). 20 mg N-ASD-C was dissolved in 2 ml dimethylformamide. This and the former solution were blended and shaken for 2-3 min until a light pink precipitate was formed. The precipitate was filtered off and the deparaffinized and hydrated slides were incubated in the resulting solution for 60 min. After incubation the slides were washed under running tap water for 10 min, rinsed in distilled water and counter-stained with Harris haematoxylin for 15 s. The slides were then washed in running tap water for 10 more min, rinsed in distilled water, dehydrated, cleared and mounted.

Evaluation of results

Mast cells were counted using a light microscope and a magnification of ×250. A grid defining an area of 0.1854 mm² was fixed to the eyepiece and the first area was adjusted to the basal cell layer of the epithelium. The first area was designated level I and the previous one levels II, III, IV and V. The method is described more in detail elsewhere (5).

The intensity of inflammation in the specimen was determined by estimating the number of inflammatory cells in the inflammatory cell infiltrate per mm². The following designations were used: - = no inflammation, + = slight inflammation, ++ = moderate inflammation and +++ = strong inflammation. The scores were converted into figures for statistical purposes as follows: - = 0, + = 1, ++ = 2, +++ = 3.

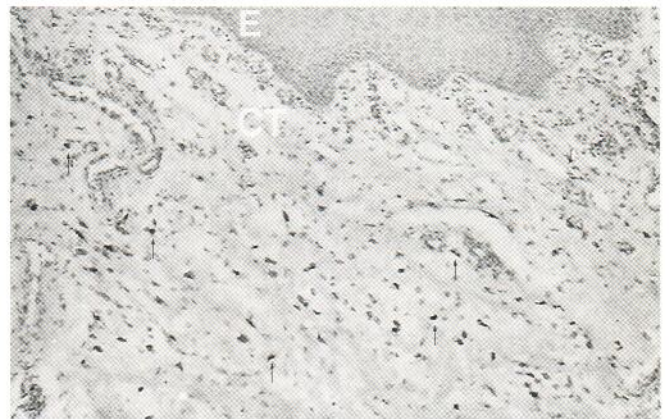


Fig. 1. Mast cells (MC) (some of them are shown with arrows) in an oral mucosal lesion of a patient with EM stained with TB. It can be seen that the number of MC increases with increasing depth into the connective tissue (ct). Original magnification ×100.

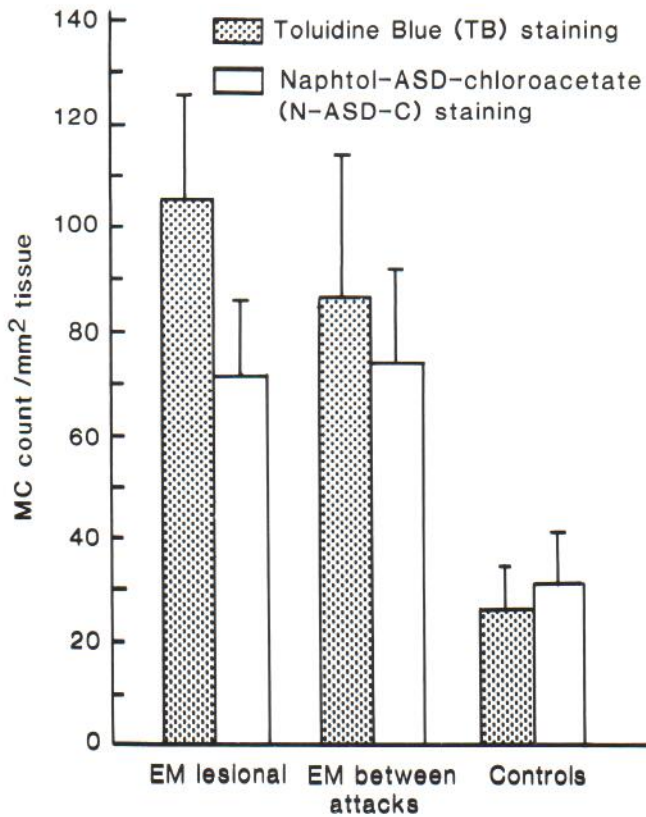


Fig. 2. Mean number and SEM of mast cells stained with TB and N-ASD-C. The differences between the groups are not statistically significant.

Statistical methods

The differences between the groups were tested using the paired t-test. The Kendall rank correlation test was used for the comparison of the two staining methods used in this study. It was also used for the comparison of the MC count and the intensity of inflammation to depth of field counted. The Spearman linear correlation test was used to compare the MC count with the intensity of inflammation.

RESULTS

After staining with TB the metachromatic mast cells appeared as violet, granular mononuclear cells (Fig. 1). Staining of non-specific esterase using N-ASD-C stained MC brownish red. MC were usually located near the blood vessels or diffusely scattered in the upper lamina propria.

MC counts from TB and N-ASD-C stained specimens correlated significantly with each other (correlation coefficient 0.80, $p < 0.001$). Lesional and normal appearing mucosa from EM patients contained more MC than control specimens, however, the differences between the groups were not statistically significant (the paired t-test) (Fig. 2).

MC counts were also made at different depths in the specimens. In lesional EM there was a slight positive correlation between the MC count and increasing depth; the highest MC counts were found in the deep mucosal layers of the lamina propria and the lowest in the superficial layers of connective tissue ($r = 0.228$, $p < 0.05$, the Kendall rank correlation test). The situation was the reverse for the specimens from healthy mucosa in patients with EM, where the MC count was highest

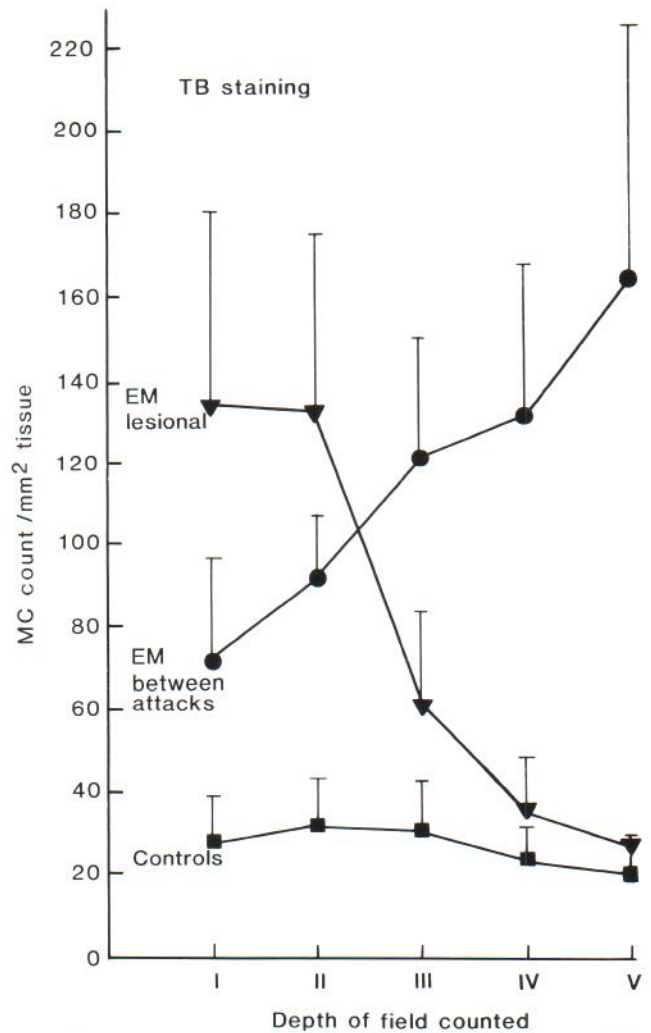


Fig. 3. MC count/mm² as a function of depth of field counted in TB staining. In EM lesions the MC count was low in superficial lamina propria, but high in normal appearing mucosa between attacks.

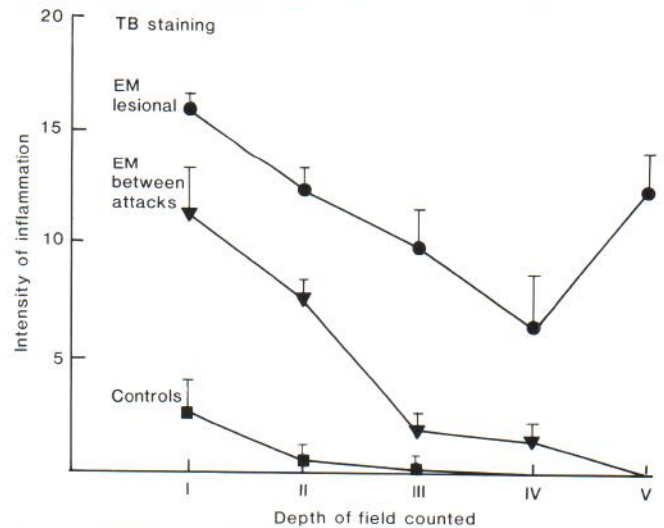


Fig. 4. Intensity of inflammation as a function of depth of field counted in TB stained samples. In lesional EM and healthy mucosa between attacks from EM patients there was a negative correlation between the intensity of inflammation and depth of field counted ($r = -0.345$, $p < 0.005$ and $r = -0.61$, $p < 0.001$, respectively, the Kendall rank correlation test).

in the superficial layers of the lamina propria, gradually decreasing to around the counts for normal mucosa from healthy volunteers ($r = -0.31$, $p < 0.05$, The Kendall rank correlation test) (Fig. 3).

The findings were also correlated with the intensity of the inflammation. In lesional EM an inverse correlation between the intensity of inflammation and increasing depth of the specimen was noticed ($r = -0.345$, $p < 0.005$, The Kendall rank correlation test). A similar but stronger inverse association was noticed in specimens from healthy mucosa in patients with EM ($r = -0.61$, $p < 0.001$, The Kendall rank correlation test). The intensity of inflammation was strongest in the fields just below the epithelium. The intensity of inflammation decreased with increasing depth into the lamina propria (Fig. 4). In lesional EM, however, level V did not show the least intense inflammation. This might be because only in three of the seven lesional EM biopsy specimens could the number of MC be counted at level V.

In lesional EM there was no correlation between the intensity of the inflammation and the MC count. In specimens from healthy mucosa in patients with EM a significant positive correlation was seen between the MC count and the intensity of the inflammation, which decreased with increasing depth into the lamina propria ($r = 0.38$, $p < 0.01$, The Spearman linear correlation test).

DISCUSSION

The use of these two staining methods, namely TB staining and N-ASD-C staining, which are based on different working principles, established beyond doubt that MC are present in oral mucosa in recurrent EM. Since N-ASD-C also stains polymorphonuclear leucocytes besides MC (4–5), counting MC was more difficult for these specimens than for TB stained samples.

The mere presence of MC does not mean that they are actively involved in local pathogenic processes. They might be responding to mitogenic stimuli, but this does not imply that they are activated in the sense that they would degranulate and generate MC mediators *de novo*. However, analysis of EM lesions disclosed no correlation between MC count and degree of inflammation. The superficial lamina propria showed the highest degree of inflammation, the lowest MC counts, a gradual decrease in inflammation intensity and a concomitant increase in TB and N-ASD-C stainable mast cells with increasing depth into the lamina propria. Paradoxical as it may sound, based on well documented works by Claman and coworkers (6–7), this phenomenon may imply MC participation, i.e. degranulation, in areas of high inflammation intensity.

The other interpretation for the low MC counts in the intensely inflamed superficial lamina propria, if the hypothesis

on degranulation and “phantom cells” as described by Claman et al. (7) is rejected, is that the total number of MC, resting or activated, is indeed low in such areas. The findings from healthy mucosa samples in patients with EM between the attacks, however, argue strongly against this: the MC count was particularly high in the superficial connective tissue. It is possible that after an acute clinical phase, when the disease goes into remission, the local inflammation gradually resolves and the “phantom MC” resynthesize their depleted granules with esterase and proteoglycan matrix content. The relatively high number of MC in those superficial connective tissue layers with low MC counts in lesional EM suggests that the first interpretation, namely local MC activation/degranulation and false negative staining is the reason for the fact that no correlation was observed between MC count and inflammation intensity.

It was also noticed that in the healthy mucosa specimens in patients with EM between the attacks, the MC count was already quite low in the deep mucosal layers of the lamina propria. This suggests that in this deep tissue, which is not affected as extensively by the disease process as the more superficial layers, the MC counts are close to those seen in normal mucosa from healthy volunteers.

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