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# Mitosis of Mast Cells in Skin Lesions of Atopic Dermatitis

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**Mitoses of mast cells with cytoplasmic granules were observed in 6 out of 55 skin lesions of atopic dermatitis. It is thus likely that the local proliferation process of mast cells contributes to the increased number of mast cells in skin lesions of atopic dermatitis. Mitotic mast cells with cytoplasmic granules were also observed in 2 out of 8 dinitrochlorobenzene contact dermatitis lesions provoked in patients with atopic dermatitis. It is suggested that some mast cells in skin lesions of atopic dermatitis might be involved in the pathogenesis of type IV eczematous skin lesions of the disease.**

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Mast cells are often increased in number in skin lesions of atopic dermatitis (1–3). But it is not clear whether the increase of mast cells is due to the accumulation of mast cell precursors in the skin from peripheral blood, or whether local proliferation of mast cells occurs in skin lesions of atopic dermatitis. Mitoses of mast cells have been described to occur in skin lesions of contact dermatitis, such as dinitrochlorobenzene (DNCB) dermatitis lesions (4, 5). Since atopic dermatitis histopathologically resembles contact dermatitis (1, 2), we tried to see, in the present study, whether or not mitotic figures of mast cells appear in skin lesions of atopic dermatitis.

## MATERIALS AND METHODS

### *Atopic dermatitis lesions*

A total of 55 patients with atopic dermatitis, who had not been treated with topical or systemic corticosteroids for at least 1 month prior to the examination, were included in the study. The diagnosis was made on the basis of morphological appearance and distribution of skin lesions, the clinical course, and the family history of atopic disease. Thus, all patients examined fulfilled Hanifin's & Rajka's criteria for atopic dermatitis (6). They ranged in age from 13 to 45 years, with a mean age of 21. One biopsy specimen was taken from each of the 19 subacute eczematous lesions, 28 lichenified patches, and 8 prurigo lesions of atopic dermatitis. The biopsy specimens were fixed with 3% glutaraldehyde and post-fixed with 1% osmium tetroxide. The specimens were then dehydrated and embedded in epon. Twenty to 50 serial 1 μm-thick sections from each biopsy specimen were prepared and stained with Giemsa solution and toluidine blue reagent.

We observed the upper dermis and perifollicular area of all the sections and counted the number of mitotic mast cells distributed in the area. The mitotic mast cell count was expressed as the number of mitotic mast cells among the total number of mast cells.

### *Allergic contact dermatitis lesions provoked in patients with atopic dermatitis*

Eight adult patients with atopic dermatitis were selected for this part of the study. The details of the study were fully discussed with each patient, and informed consent was obtained. A solution consisting of 0.1 ml of 1% DNCB in acetone on an occlusive patch test was applied to uninvolved skin for sensitization. The patch was removed after 24 h. Two weeks later, 0.1 ml of 0.1% DNCB challenge test solution was applied to normal-appearing skin (7). A biopsy specimen was then taken from DNCB dermatitis lesions. The period between the application of DNCB challenge test solution and the biopsy in each patient varied from 2 to 7 days. The biopsy specimens were immediately fixed in periodate-lysine-paraformaldehyde for 4 h (8). Sliced sections were incubated overnight with anti-IgE monoclonal antibody (Janssen Biochimica, Beerse, Belgium) at 4°C, rinsed in PBS 7% sucrose, and incubated for 2 h with preformed avidin-biotin complex (Vector Lab, Burlingame, U.S.A.). After additional fixation in 2% glutaraldehyde, the reaction product was revealed by incubation in PBS, pH 7.6, 0.03% diaminobenzidin, and 0.01% $H_2O_2$ for 10 min at room temperature (9). The specimens were post-fixed with 1% osmium tetroxide, dehydrated by ethanol and embedded in epon. Twenty to 50 semi-thin 1 μm sections were prepared from the epon blocks and counterstained using 0.02% toluidine blue. Ultrathin sections were stained with uranyl acetate and lead citrate and observed with a Hitachi H-600 and a Philips CM-12 electron microscope. We observed the upper dermis and perifollicular area in all of the semi-thin sections and ultrathin sections. The mitotic mast cell count was similarly expressed as the actual number of mast cells out of the total number of mast cells distributed in the areas.

### *Controls*

Twenty to 50 semi-thin 1 μm sections were prepared from each biopsy specimen taken from a non-eczematous skin lesion in 6 patients with atopic dermatitis and normal-appearing skin in 5 non-atopic volunteers. The semi-thin sections were stained with 0.02% toluidine blue.

Table I. *Frequency of occurrence of mitotic mast cells in the different types of skin lesions of atopic dermatitis, contact dermatitis, non-eczematous skin in patients with atopic dermatitis and in normal-appearing skin in non-atopic volunteers*

| | Biopsy sites in patients with atopic dermatitis | | | | | Biopsy sites in non-atopic volunteers |
|---|---|---|---|---|---|---|
| | Skin lesions of atopic dermatitis | | | DNCB dermatitis lesions | Non-eczematous skin | Normal-appearing skin |
| | Subacute lesions | Lichenified lesions | Prurigo lesions | | | |
| No. of patients | 19 | 28 | 8 | 8 | 6 | 5 |
| No. of mast cells observed | 9450 | 10335 | 7520 | 3020 | 2955 | 1051 |
| No. of mitotic mast cells | 2 | 3 | 1 | 2 | 0 | 0 |

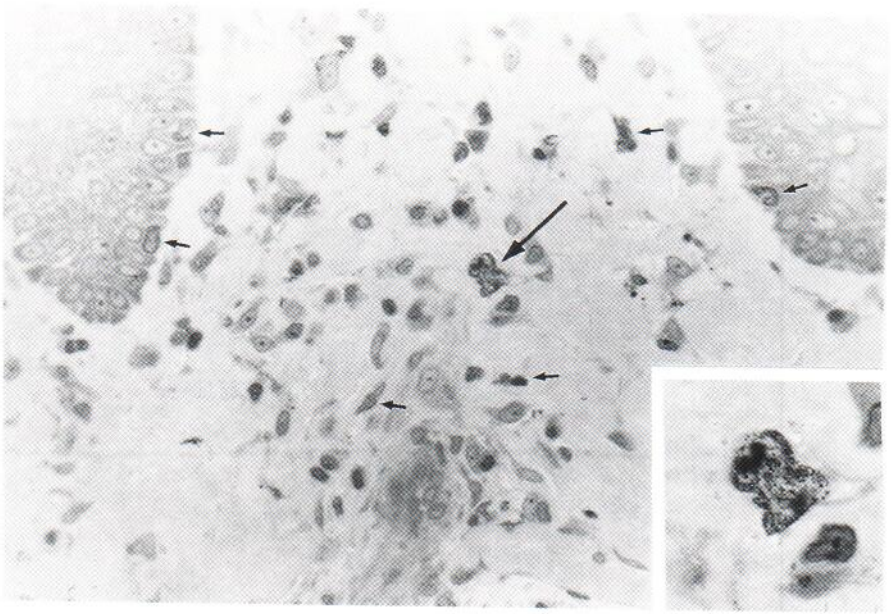

*Fig. 1.* Atopic dermatitis lesion. One μm-thick semi-thin section. Giemsa staining. A mitotic mast cell (*large arrow*) is seen in the upper dermis and many mast cells (*small arrows*) are observed in the dermis and epidermis. *Inset:* a mitotic mast cell with cytoplasmic granules.

The distribution of mitotic mast cells was examined by the same method as described above.

## RESULTS

### *Atopic dermatitis*

Mitotic figures were observed in 2 out of 19 subacute lesions, 3 out of 28 lichenified lesions, and 1 out of 8 prurigo lesions of atopic dermatitis (Table I). The actual number of mitotic mast cells was, however, very small, as shown in Table I.

The mitotic mast cells had many metachromatic granules in cytoplasm that were similar in color to those of non-mitotic mast cells (Fig. 1). Fig. 2 presents serial sections of a mitotic mast cell with cytoplasmic granules, showing spatial conformation of cytoplasm, chromatin, and mast cell granules.

### *Allergic contact dermatitis lesions provoked in patients with atopic dermatitis*

Mitotic figures of mast cells with cytoplasmic granules were seen in 2 out of 8 DNCB dermatitis lesions provoked in patients with atopic dermatitis (Table I). The actual number of mitotic mast cells was very small. In the electron microscope (Fig. 3), chromosomes were observed in the center of the cytoplasm and a small number of mast cell granules were distributed in the periphery. The cell membrane of the mitotic mast cells was positively stained with anti-IgE antibody.

### *Controls*

Mitotic mast cells were absent in non-eczematous skin of 6 patients with atopic dermatitis, and in normal-appearing skin of 5 non-atopic volunteers (Table I).

## DISCUSSION

The present study demonstrates that mitosis of mast cells with cytoplasmic granules occurs in skin lesions of atopic dermatitis. Mitoses of connective tissue mast cells have been reported to occur mostly in the dedifferentiation process of mast cells

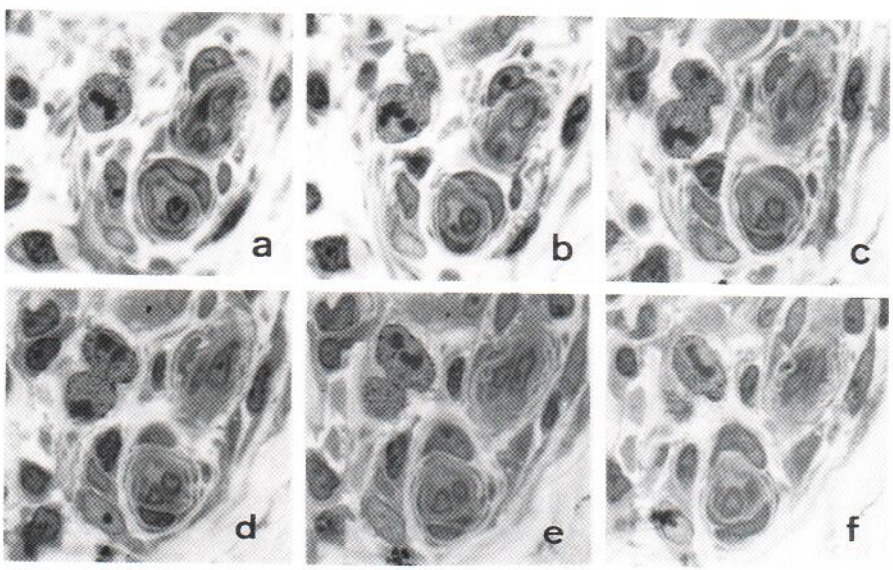


*Fig. 2.* Atopic dermatitis lesion. One μm-thick semi-thin section. Giemsa staining. Serial sections of a mast cell are shown. Spatial conformation of cytoplasm, chromosomes, and mast cell granules is observed.

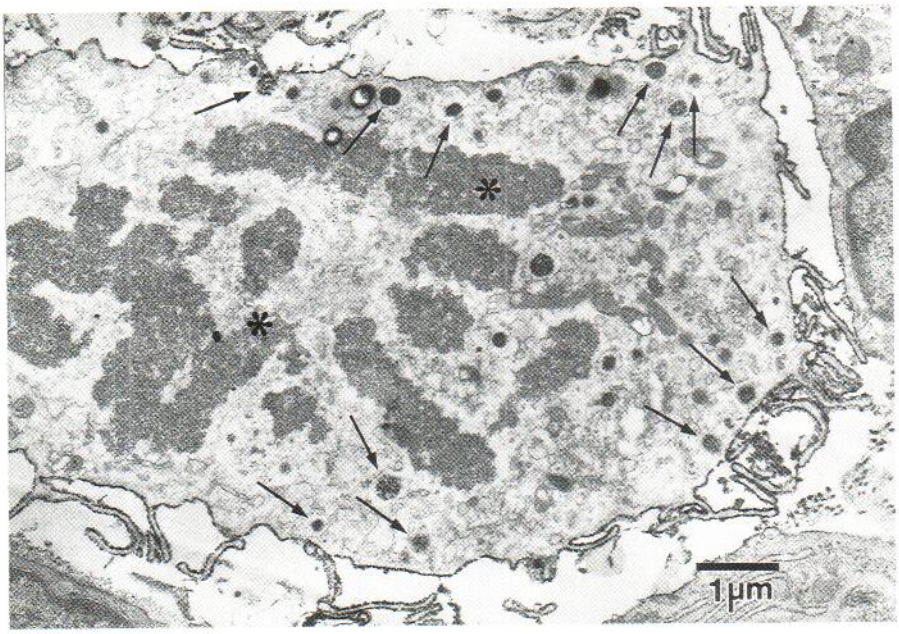


*Fig. 3.* DNCB dermatitis lesions of a patient with atopic dermatitis. IgE immune electron microscopy. A mitotic mast cell with chromosomes (*) and mast cell granules (*arrows*) are seen in the cytoplasm. Cell membrane of the mast cell is positively stained with anti-IgE antibody.

(10). The dedifferentiated mast cells cannot be morphologically identified as mast cells because mast cells in the dedifferentiation process do not have cytoplasmic granules (10). Thus, although the occurrence of mitoses of mast cells with cytoplasmic granules in skin lesions of atopic dermatitis was very rare, the total number of mitotic mast cells might be larger than that observed in the present study. It is likely that a local proliferation of mast cells may contribute at least in part to the increased number of mast cells in skin lesions of atopic dermatitis. It has been reported that mast cells in mice are derived from stem cells in the bone marrow, which are transformed into circulating mast cell precursors before they settle in the skin (10). Thus, an abundant settlement of mast cell precursors may also contribute to the increase of mast cells in skin lesions of atopic dermatitis.

Mechanisms that provoke mitosis of mast cells in skin lesions of atopic dermatitis are speculative. Activated inducer T lymphocytes release interleukin-3, which stimulates proliferation of mast cells in mice (11, 12), though in humans, interleukin-3 induces basophil rather than mast cell differentiation (13). Recently, it has been shown that CD34+ cells cultured in the presence of both human interleukin-3 and stem cell factor give rise to human basophils and mast cells (14).

The present study further shows that mitosis of mast cells with cytoplasmic granules occurs in DNCB dermatitis lesions provoked in patients with atopic dermatitis. This is in agreement with the findings of Dvorak et al. (4, 5) that mitotic mast cells with cytoplasmic granules appear in DNCB dermatitis lesions in healthy individuals. According to Dvorak et al. the presence of mitotic mast cells indicates that mast cells might play an important role in the production of delayed skin reaction in DNCB dermatitis. Various authors state that T-cell dependent activation of mast cells plays a role for the elicitation of delayed hypersensitivity responses (15–20). Skin lesions of atopic dermatitis, histologically and immunohistologically, have features of type IV eczematous reaction (21-23). According to Dvorak's rationale (4, 5), the existence of mitotic mast cells in skin lesions of atopic dermatitis seems to indicate that some mast cells in skin lesions of atopic dermatitis might be involved in the pathogenesis of eczematous skin lesions of the disease.

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