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SHORT COMMUNICATION

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Mast cell chymase is increased in chronic atopic dermatitis but not in psoriasis

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Abstract Mast cell chymase is a chymotrypsin-like serine proteinase primarily stored in secretory mast cell granules. Mast cell chymase has various effects on angiotensin, metalloproteases, lipoproteins, procollagen, neuropeptides and cytokines. Recent studies have demonstrated that chymase inhibitors inhibit skin inflammation. In this study we sought to determine the role of mast cell chymase in atopic dermatitis (AD) in comparison with its role in psoriasis and normal skin. Skin biopsy specimens were obtained from non-lesional and lesional skin of patients with chronic AD and psoriasis and from normal skin of non-atopic and non-psoriatic controls. The number of mast cells containing chymase was determined by immunohistochemistry using a chymase-specific monoclonal antibody. A significantly ($P < 0.05$) enhanced number of chymase-positive cells was found in lesional AD skin as compared to normal skin as well as to lesional and non-lesional skin of patients with psoriasis. A significant ($P < 0.05$) increase in the number of chymase-positive cells was also found in non-lesional AD skin in comparison to psoriasis. An enhanced, albeit not statistically significant difference was noted in non-lesional AD skin as compared to normal skin. In conclusion, these results suggest that mast cell chymase may play an integral part in eliciting and maintaining cutaneous inflammation in AD but not in psoriasis. The increased proteinase activity of mast cell chymase may also be involved in promoting a skin barrier defect in AD, which subsequently enhances the skin's permeability to allergens and microbes and thereby aggravates the eczema.

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Introduction

Atopic dermatitis (AD) is a chronic inflammatory skin disease characterized by extreme pruritus, relapsing eczematous skin lesions and a personal or family history of atopic diseases. Besides genetic, environmental and pharmacological factors, various immunological mechanisms involving both the innate and adaptive immune systems are thought to be critical in the pathogenesis of AD [1]. Mast cells are an important component of innate immunity and increased numbers of these cells are observed in chronic AD skin lesions [2]. Mast cell chymase (MCC) is a chymotrypsin-like serine proteinase which is typically stored together with other proteinases such as tryptase in secretory mast cell granules. The main effects of MCC include processing of various substances such as angiotensin, metalloproteases, lipoproteins, procollagen, neuropeptides and cytokines [3]. Whereas polymorphism in the MCC gene has been reported to be associated with AD by Mao et al. [4], other investigations have failed to confirm this association [5, 6]. Nevertheless, chymase inhibitors have recently been shown to inhibit skin inflammation and an important role for MCC in inducing inflammatory skin responses has also been indicated in animal models of AD [3]. In order to further clarify the role of MCC in eliciting and maintaining eczema, we investigated immunoreactivity for chymase in non-lesional and lesional skin of patients with AD in comparison to that in skin of psoriatic patients and non-atopic and non-psoriatic controls.

Materials and methods

A group of 19 Caucasian patients (ten females and nine males, age range 20–52 years) with moderate to severe

chronic AD were included in the study after providing informed consent. AD was diagnosed according to the criteria of Hanifin and Rajka [7]. Punch biopsy specimens of diameter 5 mm were taken from chronic eczematous skin lesions ($n = 8$) in the large joint flexures, which typically showed signs of lichenification, some hyperkeratosis, erythema and excoriations, and from non-lesional skin ($n = 11$) located about 10 cm from a chronic eczematous skin lesion. In order to compare the immunoreactivity for chymase with another inflammatory skin disease, punch biopsy specimens were also obtained from lesional ($n = 8$) and non-lesional skin ($n = 7$) of patients with psoriasis. None of these patients had received any systemic corticosteroids or applied topical corticosteroids at the site of the biopsy specimens for at least 3 weeks prior to the investigation. Furthermore, normal skin from non-atopic and non-psoriatic subjects ($n = 11$) and one patient with cutaneous mastocytosis was taken as a control.

The material was snap-frozen with or without tissue-embedding medium using isopentane precooled in liquid nitrogen and stored at −70°C. Immunostaining for MCC was performed using the avidin-biotin-complex/alkaline phosphatase (ABC/AP) method. Cryostat tissue sections of thickness 6 μm were air-dried, fixed in acetone for 8 min and rehydrated in Tris-buffered saline with 0.1% saponin. The sections were then incubated for 3 h with a monoclonal mouse anti-human MCC antibody (1.0 mg/ml, dilution 1:700, clone CCI; Serotec, Oxford, UK). After washing, the sections were incubated for 1 h with a rabbit antimouse antibody (E0413; DakoCytomation, Glostrup, Denmark) and thereafter with ABC/AP (K0376; DakoCytomation). Finally, all sections were developed with fuchsin (K0624; DakoCytomation) and counterstained with hematoxylin. Substitution of the primary antibody with isotype-matched IgG and omission of the primary antibody were used as negative controls. Skin sections from a patient with cutaneous mastocytosis were also stained as a positive control in order to validate the specificity of the staining.

The slides were analyzed under a Leitz Dialux 20EB microscope by two independent investigators. In each section, staining was assessed on 15–25 fields at ×400 magnification. Cells displaying a strong cytoplasmic staining and a nucleus were counted. The cells were evaluated using a 0.063 mm^2 grid and the number of positive cells (mean ± SEM) per millimeter squared was determined. Statistical analysis was performed using the Mann-Whitney U test (with the Bonferroni correction). P values < 0.05 were considered statistically significant.

Results

Quantification and localization of MCC-positive cells are shown in Figs. 1 and 2. A significantly ($P < 0.05$) enhanced number of MCC-positive cells was found in lesional AD skin (Fig. 1a) as compared to normal skin from non-atopic and non-psoriatic subjects (Fig. 1c) and compared to lesional and non-lesional skin of patients with psoriasis (Fig. 1d, e). A statistically significant ($P < 0.05$) increase in the number of MCC-positive mast cells was also found in non-lesional AD skin (Fig. 1b) in comparison to psoriatic skin. An enhanced, but not significant, increase in the number of MCC-positive mast cells was noted in non-lesional AD skin as compared to normal skin from non-atopic and non-psoriatic subjects. Immunoreactivity for MCC was significantly decreased in non-lesional and lesional psoriatic skin as compared to normal skin. In lesional AD skin immunoreactivity for MCC was mainly localized around the superficial dermal vascular plexus (Fig. 1g) and, although less pronounced, along the dermoepidermal junction zone (Fig. 1f).

Discussion

In this study immunoreactivity for MCC was analyzed in skin from patients with chronic inflammatory skin diseases, namely AD and psoriasis, and compared with that in normal skin. The number of mast cells containing chymase was significantly increased in chronic skin lesions of patients with AD but not in psoriatic skin. A tendency to an enhanced, albeit statistically not significant, number of mast cells containing chymase was also seen in non-lesional AD skin. Using a different enzyme-histochemical method, Järvikallio et al. [8] had in fact previously demonstrated a significantly enhanced expression of MCC in non-lesional AD skin as well as acute/subacute AD lesions. The use of a more stringent non-parametric statistical analysis in our study may partly explain this difference.

The increased expression of MCC in AD provides an explanation for some of the typical clinical features of patients with AD such as facial pallor and the tendency to develop severe chronic eczema on the face and hands. Interestingly, Weber et al. have recently reported that mast cell numbers in humans are markedly increased at peripheral anatomical sites such as the face and the distal parts of the extremities, particularly the hands and feet [9]. Since MCC can convert angiotensin I to angiotensin II [10], the vasoconstrictive property of this peptide may at least partly explain the facial pallor which is seen in up to 50–60% of AD patients.

Previous studies have demonstrated that MCC has a range of important proinflammatory effects [3]. Mizutani et al. [11] have reported that MCC leads to a rapid and specific conversion of precursor interleukin-1β (IL-1β) to active IL-1. Furthermore, injection of chymase has been shown to cause an inflammatory response in the skin, which is inhibited by different chymase inhibitors [12]. Taking together the increased expression of MCC in AD and the enhanced numbers of mast cells in the hands and the face [9], one may speculate that

MCC is particularly a critical factor in eliciting and perpetuating eczema at these body sites in patients with AD. Enhancement of MCC seems rather specific for the type of inflammatory skin response found in AD, since no increase in the number of mast cells containing chymase was seen in psoriasis. Although the precise reasons for these distinctions still remain to be elucidated, a different cytokine/chemokine milieu in these two chronic inflammatory skin disorders may be responsible for the increased number of mast cells containing chymase in AD [13]. A previously reported increase in the expression of proteinase inhibitors in psoriasis provides an additional explanation [14]. Such proteinase inhibitors lead to conformational changes of chymase, which not only inhibit the activity of this protein but may also influence the binding affinity and thereby the immunoreactivity of the monoclonal antibody (CC1) used for the detection of chymase in this study.

Structural changes, including papillary dermal fibrosis and epidermal hyperplasia are further features of chronic AD [1]. Besides various cytokines MCC may also play an important part in the induction of tissue fibrosis and matrix remodeling due to its known effects on activation of procollagenase and metalloproteases as well as degradation of extracellular matrix proteins [3].

Furthermore, dry skin together with itch are often initially involved in triggering AD. Indeed, previous reports have shown that chymase is a possible candidate for mediating mast cell-induced pruritus in AD [15]. Moreover, previous studies have suggested that chy-

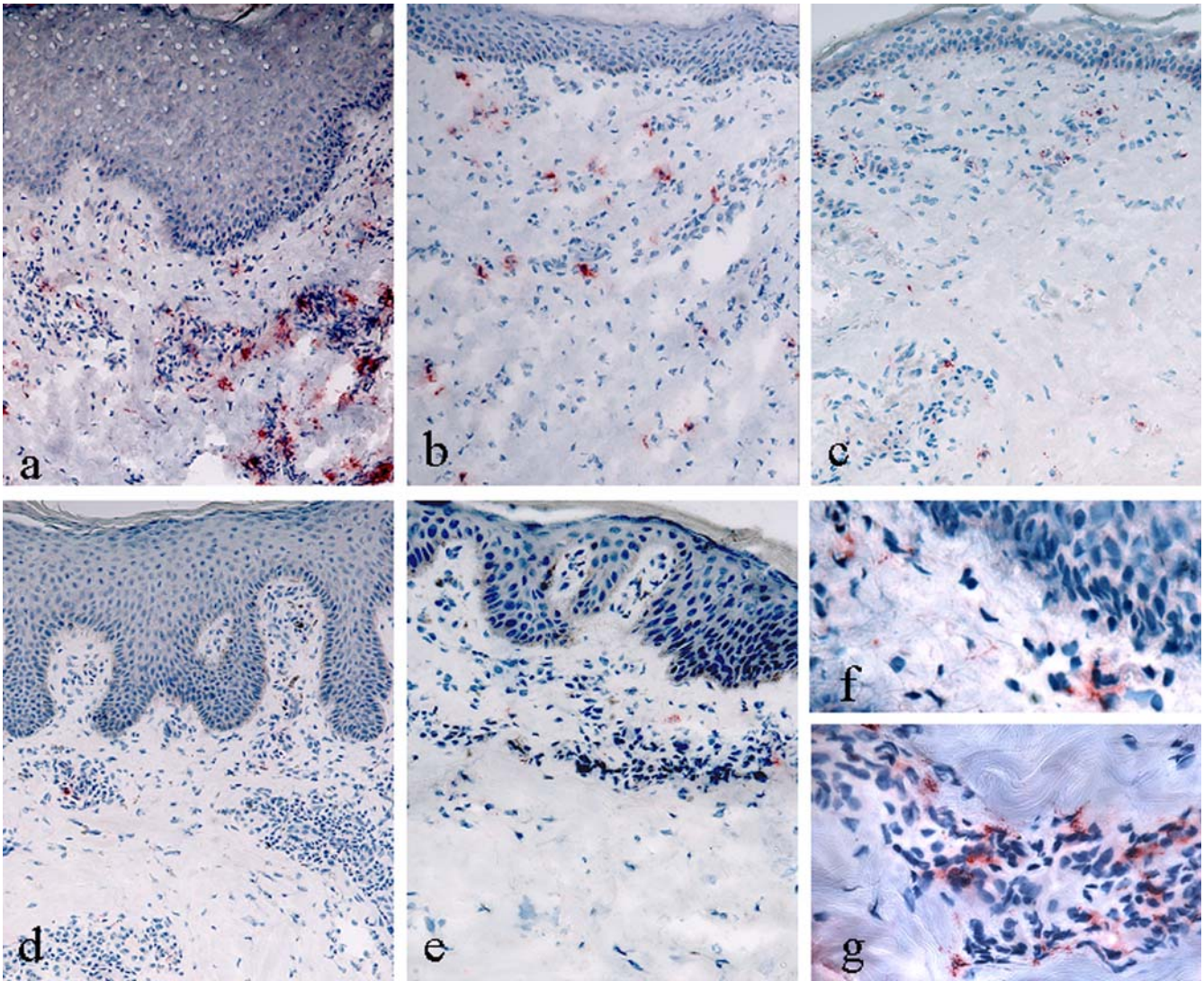

Fig. 1 Immunoreactivity for MCC is markedly increased in AD. Skin sections from lesional and non-lesional skin of patients with AD and psoriasis and one non-atopic, non-psoriatic subject are shown as representative examples. An increased number of mast cells containing chymase was observed in the dermis of lesional (**a**) and non-lesional (**b**) AD skin in comparison to the non-atopic, non-psoriatic control (**c**) as well as lesional (**d**) and non-lesional (**e**) psoriatic skin (ABC/AP method, original magnification ×100). In lesional AD skin immunoreactivity for MCC was mainly localized around the superficial dermal vascular plexus (**g**) and, although less pronounced, along the dermoepidermal junction zone (**f**) (ABC/AP method, original magnification ×400)

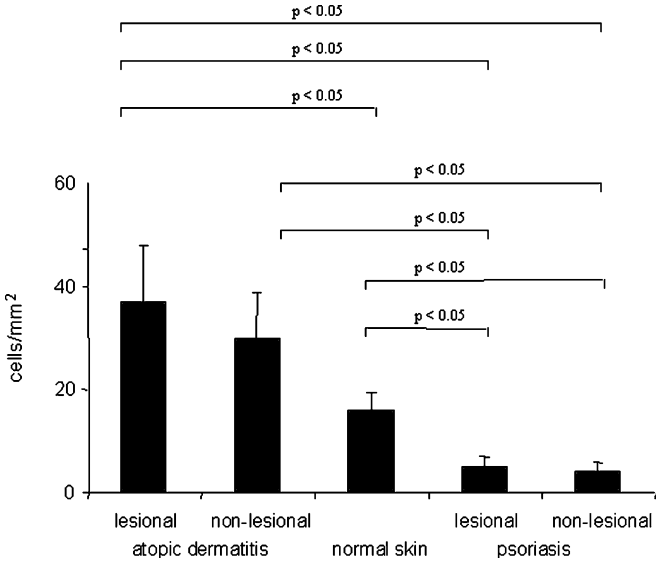

Fig. 2 Quantification of MCC in lesional and non-lesional skin of patients with AD and psoriasis and in normal skin. The data presented are means ± SEM. The results of the statistical analysis (Mann-Whitney *U* test) are indicated

motrypsin-like proteinases can modify proteinase-activated receptors, which are present on keratinocytes and are thought to be involved in epidermal growth and repair processes [16, 17]. Although the precise effects of MCC on keratinocytes in AD still remain to be elucidated, MCC may participate in the events leading to skin barrier dysfunction and dry skin. Interestingly, recent reports have demonstrated that Netherton syndrome, which is characterized by ichthyosis associated with erythroderma, hair shaft defects and atopic features are caused by a mutation in the gene (SPINK5) encoding the serine proteinase inhibitor LEKT1 [18]. The lack of LEKT1 is thought to result in increased protease activity in the stratum corneum, accelerated degradation of desmoglein-1, and finally increased desquamation of corneocytes leading to hyperkeratosis. This may lead to the reduced lipid content in the stratum corneum and/or altered composition from incomplete lipid processing [18]. Intriguingly, a significantly decreased expression of LEKT1 precursor has recently also been reported in AD in comparison to psoriasis [13]. Thus, it is tempting to speculate that increased amounts of proteinases like MCC primarily lead to an epidermal defect that increases the skin's permeability to allergens and microbes which subsequently aggravate the eczema.

In conclusion, the increased number of mast cells containing chymase in AD points to a critical role for chymase in initiating as well as maintaining eczema in these patients. The precise pathophysiological relevance of chymase in eliciting skin barrier dysfunction in AD remains to be clarified in future studies.

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