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Macrophage migration inhibitory factor (MIF) in the stratum corneum: A marker of the local severity of atopic dermatitis

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Abstract

Different biomarkers are used to evaluate the severity of atopic dermatitis (AD); however, it remains

difficult to determine the severity of localized skin lesions. MIF plays an essential role in the
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pathophysiology of skin inflammation. In order to establish whether the MIF level in the stratum corneum (SC) serves as a marker of the severity of AD lesions, we examined the SC MIF (scMIF) levels in AD patients. The SC of the cheek, neck and upper arm skin was collected using tape stripping, and the scMIF levels were measured. Consequently, the scMIF levels were found to be significantly higher in the involved skin lesions than the uninvolved areas within the same patient. Moreover, the scMIF levels were significantly correlated with the severity of local skin lesions. These findings suggest that the scMIF level can be used as an effective marker for evaluating the local severity of AD.

Key words: macrophage migration inhibitory factor • atopic dermatitis • stratum corneum • tape-stripping • SCORAD

Background

Atopic dermatitis (AD) is a chronically relapsing inflammatory skin disease. The dysregulation of various inflammatory cytokines and growth factors secreted by keratinocytes and peripheral mononuclear cells is a major cause of AD (1, 2). Skin barrier disruption is another contributing factor in AD (3, 4). Macrophage migration inhibitory factor (MIF) functions as a pleiotropic cytokine by participating in the processes of inflammation and immune responses (5) and thus contributes to the immunoregulation and pathophysiology of allergic diseases. Indeed, a high MIF expression has been found in a variety of inflammatory disorders, including AD and inflammatory skin lesions (6-8).

In order to provide the best clinical treatment for AD patients, it is important to correctly evaluate the clinical characteristics and conditions of affected individuals. Currently, the serum level of thymus and activation-regulated chemokine (TARC) is measured to evaluate the severity of AD. However, the development of a non-invasive method of assessing AD is desirable in order to avoid

the need for a skin biopsy or blood draw. Recently, the TARC levels in the stratum corneum (SC) were found to be increased in the skin lesions of AD patients, in correlation with the disease severity (9). Successful AD management requires an accurate assessment of the features of local skin lesions in order to effectively evaluate the patient's condition and prescribe the most suitable treatment. Therefore, it is important to identify new biomarkers that better reflect the degree of local inflammation in AD skin lesions.

Questions addressed

In this study, we measured the MIF levels in tape-stripped SC samples obtained from AD patients and evaluated the correlation between the SC MIF (scMIF) level and the local skin severity.

Experimental design

Subjects and evaluation of clinical features

The study subjects included 43 patients with AD (28 males and 15 females; age, 18–51 years; mean age, 30.0 years). The diagnosis of AD was made based on the Rajka and Hanifin criteria (10), and the patients had no other concomitant diseases. Twenty healthy volunteers (nine males and 11 females; age, 22–41 years, mean age, 28.2 years) with no current or past history of atopic disease were also enrolled. The study was approved by the Ethics Committee of the University of Toyama. The SCORAD index was used to evaluate the severity of AD (11), and the local AD severity score in the involved skin was measured according to the scores for erythema, edema/papules, oozing/crust formation, excoriation, lichenification and xerosis, each of which was assessed on a four-point scale (12).

Preparation and measurement of the SC and blood samples

SC samples were obtained according to the tape-stripping method using skin tape (Harney Layer

Checker; Asahi Biomed, Tokyo, Japan). The tape was attached to a skin lesion on the AD patient's cheek, neck or upper arm and carefully pressed with a finger then detached. Similarly, a control SC sample was obtained from a close region of uninvolved skin in the AD patients and healthy subjects. Blood samples were obtained to determine the levels of white blood cells, eosinophils, serum IgE, LDH and TARC. The serum MIF levels were measured using an ELISA kit specific for MIF (Sapporo Immuno diagnostics laboratory, Sapporo, Japan). The MIF levels in the SC samples obtained via tape stripping were measured using an ELISA kit specific for MIF (R&D systems, Minneapolis, MN). The Steel-Dwass test or the Pearson product-moment correlation test were used in this study.

Results

The characteristics of the enrolled patients with AD are shown in **Table S1**. The serum MIF levels of the AD patients (12.3 ± 2.6 ng/ml) were significantly higher than those of the healthy controls (5.3 ± 0.5 ng/ml) ($p=0.01$).

The scMIF levels obtained from the cheek, neck and upper arm skin were compared between the AD patients and healthy controls. The scMIF levels in three areas were significantly higher in the involved skin lesions than in the healthy control samples ($p<0.01$) (**Figure 1**). Furthermore, the scMIF levels in the involved skin lesions were significantly higher than those observed in the uninvolved areas obtained from the same subject among the AD patients ($p<0.01$).

We then evaluated the correlation between the scMIF level and the local disease severity in the cheek, neck and upper arm. The results showed that the scMIF level was significantly correlated with the severity of the local skin lesion at each skin site ($p=0.0001$) (**Figure 2**).

Discussion and Conclusion

Increased production of inflammatory cytokines and growth factors, such as IL-5, IL-6, IFN- γ , TNF- α , GM-CSF and MIF, has been reported in AD skin lesions (13). The present study demonstrated that the scMIF levels were significantly higher in the involved lesions than the uninvolved skin or SC samples obtained from the healthy subjects. MIF proteins are generally limited to the basal layer in normal skin (14, 15). However, they are diffusely distributed throughout the involved epidermal layer in AD patients with hyperplasia (7). Our findings indicate that a high production of scMIF reflects the high expression of MIF observed in the keratinocytes present in the involved skin in AD patients.

In the current study, the scMIF levels in the cheek, neck and upper arm skin were correlated with the severity of the local skin lesions. Facial dermatitis, a form of AD that affects the cheek and head, characterized by erythema, itching, weeping lesions and crust formation, is often resistant to conventional therapy. Therefore, we speculate that the scMIF level can be used as an effective marker for evaluating the local skin severity in AD patients. In support of our observations, the expression levels of thymic stromal erythropoietin, an IL-7-like cytokine, are increased in the SC samples of AD patients compared to that observed in healthy subjects and correlate with the SCORAD score (12). This bioactive marker plays an important role in the pathogenesis of skin diseases. In the near future, additional cytokine markers may be analyzed in combination with MIF in order to evaluate AD symptoms in greater detail.

In conclusion, our findings suggest that the scMIF level reflects the local severity in the inflammation phase in AD patients.

Author contributions

CY, SI, SM and TS designed the experiments; NM, KK, KM and YY performed most of the research; CY, AE and SI analyzed and interpreted the data; CY, YY, SM and TS wrote the paper; all authors revised and approved the final manuscript version.

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Conflict of interests

The authors report no conflicts of interest.

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rat skin during embryonic development. *Exp Dermatol* 2005; **14**: 819-823.

Figure Legends

Figure 1. The stratum corneum (sc) MIF levels in the AD patients and healthy controls. The MIF content (pg/ μ g protein) was measured in the SC samples obtained from the involved and uninvolved areas in the AD patients (n= 43) and normal healthy controls (n=20). (a) Cheek, (b) neck, (c) upper arm. The Steel-Dwass test was used to compare the MIF levels between the AD patients and healthy control subjects. The data are expressed as the mean $\pm$ standard error of the mean. *p< 0.01

Figure 2. Correlation between the scMIF levels in the involved areas and the local severity scores in the AD patients. The correlation between the MIF levels in the SC samples and the local disease severity was analyzed according to the Pearson product-moment correlation test, in which a p value of <0.01 was considered to be statistically significant. (a) Cheek: n=42; (b) neck: n=36; (c) upper arm: n=39.

Figure 1

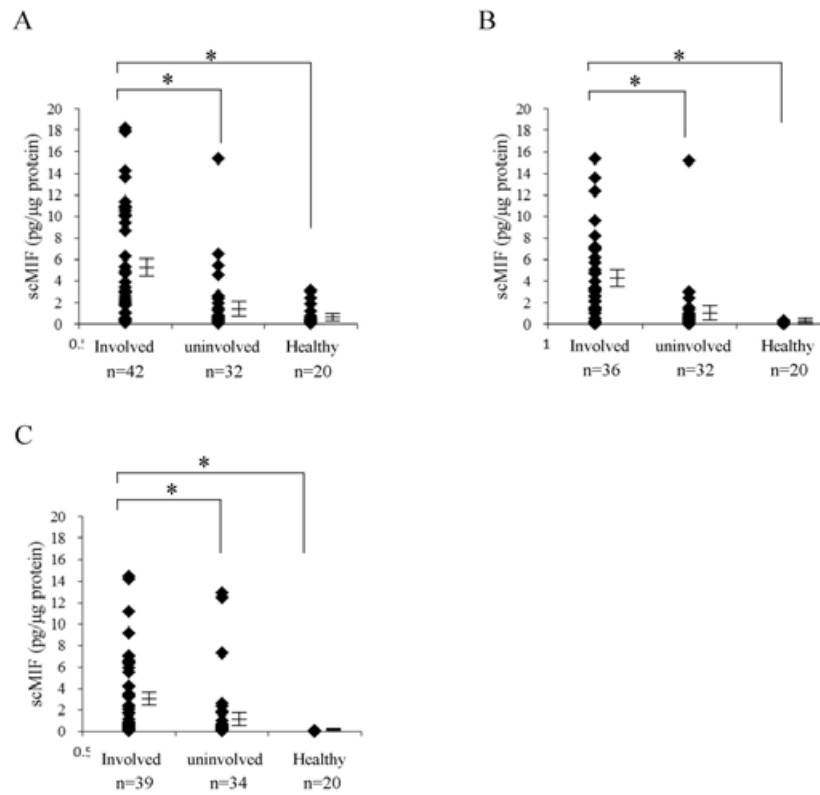


Figure 2

