学 位 論 文 の 要 旨

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学 位 論 文 名　Interleukin-8 Content in the Stratum Corneum as an Indicator of the Severity of Inflammation in the Lesions of Atopic Dermatitis

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論 文 内 容 の 要 旨

INTRODUCTION

Atopic dermatitis (AD) is a relapsing or chronic skin disease that is characterized by highly pruritic eczema. The prevalence of AD has increased in the last half century, but the mechanisms involved in the initiation and maintenance of skin inflammation in the AD are not fully understood. Recent studies have provided insights into the underlying immunologic mechanisms, suggesting an amplification cycle of atopic skin inflammation. Investigations of AD in patients and animal models suggest that this disease is initiated, maintained and perpetuated by the actions of various inflammatory cytokines and growth factors secreted by keratinocytes, antigen-presenting dendritic cells, T cells and other inflammatory cells, although there is also evidence of skin barrier defect. These cytokines and growth factors regulate not only the immune and inflammatory responses but also the proliferation and differentiation of skin components, thus these components can reflect inflammatory conditions of lesional skin in AD. We previously reported a method for assessing the severity of individual lesions to measure TARC levels in lesional stratum corneum obtained by tape-stripping. In our next study, we measured the amount of vascular endothelial growth factor (VEGF) in the stratum corneum obtained by type-stripping with specific ELISA directly, and found that the amount of VEGF is well correlated with the severity of the acute phase inflammation, such as erythema and edema, but not correlated with the chronic phase of inflammation of lesional skin of AD. In this study, in order to establish a means of evaluating the severity of individual lesions, the levels of IL-8, IL-18, VEGF and TGF-α in the stratum corneum (scIL-8, scIL-18, scVEGF and scTGF-α) were

evaluated on various skin sites in the patients with AD and examined the correlation between the levels of lesional cytokines and the clinical severity scores of the corresponding lesions.

MATERIALS AND METHODS

Fifty patients with AD who fulfilled the diagnostic criteria for AD established by the Japanese Dermatological Association and a total of 12 healthy subjects were enrolled in the study. The stratum corneum was collected from the healthy subjects and patients with AD by using the tape-stripping technique on the skin of the forearm, neck and back. The clinical severity of AD was evaluated as slight, mild, moderate or severe according to the diagnostic criteria for the severity scoring of atopic dermatitis (SCORAD) index. The SCORAD index was calculated by the equation A/5 + 3.5B + C, where A is the percentage of body area with a skin rash; B is the total score of erythema, edema/papules, excoriation, oozing/crust, lichenification and xerosis, each of which was evaluated on a 4-point scale (0: none, 1: mild, 2: moderate or 3: severe), and C is the sum of the visual analog scale (a scale ranging from 0 to 10), which was used in the subjective assessment of both itching and sleep loss (average for the last 3 days), and in this study was as follows: 22 patients had slight AD, 10 patients had mild AD, 11 patients had moderate AD and 7 patients had severe AD. Manifestation scores of skin lesion were evaluated according to B score. Trans-epidermal water loss (TEWL) and skin water content (SWC) of the lesions were also measured prior to tape-stripping. The amounts of IL-8, IL-18, VEGF and TGF-α were measured using specific ELISA kits. Forty seven patients with AD were enrolled in the blood tests. Serum levels of total IgE, lactate dehydrogenase (LDH) and peripheral blood eosinophil count were measured by laboratory tests or serum TARC was measured by ELISA.

RESULTS AND DISCUSSION

The levels of scIL-8, scIL-18, scVEGF and scTGF-α from the arm, neck and back region were significantly higher in the AD patients as compared to those of healthy controls. The cytokines levels of both the unaffected and affected areas of AD patients were significantly higher than those of healthy controls. The levels of scIL-8 and scIL-18 were significantly higher in the affected versus the unaffected areas. When the levels of the cytokines were compared among the patient groups divided according to disease severity, the increase in scIL-8 was shown to be directly proportional to the severity of AD. The correlation of scIL-8, scIL-18, scVEGF and scTGF-α with the severity of local inflammation was assessed using the manifestation scores of skin lesions in AD patients. The scIL-8 level correlated highly with the scores for acute and chronic AD lesions. The scIL-18 level correlated highly with the scores for acute AD lesions and weakly correlated with the scores for chronic AD lesions. A significant correlation was also

observed between scVEGF level and scores of acute AD lesions. No correlation was seen between scTGF-α and the manifestation scores of the lesions in AD. The levels of scIL-8 and scIL-18 in all the sites correlated positively with TEWL and negatively with SWC. We found scIL-8 level to have a positive correlation with serum LDH, serum total IgE, serum TARC, eosinophil count and basophil count, and significant negative correlation with lymphocyte count. In contrast, scTGF-α level did not correlate with serum levels of any of the laboratory parameters at any site.

In this study, we established that the scIL-8 level in the lesions of AD is best correlated with the severity of lesions among a variety of cytokines and growth factors. IL-8 has originally been extracted from the scales obtained from psoriasis lesions and is found to be a potent chemotactic factor for neutrophils, thus considered to play an important role in inflammatory processes of the skin with sterile neutrophil infiltration, such as psoriasis. Our study suggests that IL-8 production in the epidermal cells is a ubiquitous process in the initiation of skin inflammation, including AD. Although IL-8 detected in tape-stripped stratum corneum is considered to derive from activated keratinocytes, it is also conceivable that a part of IL-8 is derived from activated leukocytes, especially in cases of spongiosis or excoriated skin. Immunohistochemical studies using anti-IL-8 antibodies have demonstrated intracellular IL-8 staining in the lesions of AD, suggesting that IL-8 plays an important role in the pathogenesis of AD by recruiting inflammatory cells. Because IL-8 is produced by cultured keratinocytes stimulated with *Staphylococcus aureus* (*S.aureus*) and scIL-18 is known to be correlated with *S. aureus* colonization, it is likely that both IL-8 and IL-18 are induced by *S. aureus* colonization through a common induction mechanism.

CONCLUSION

The scIL-8 levels are strongly correlated with the severity of individual lesions, particularly the degree of erythema, edema/papules and excoriation. Additionally, a tape-stripping method is useful to evaluate the scIL-8 in combination with the IL-8 specific ELISA. Thus scIL-8 might be utilized to evaluate precisely the effect of new treatment tools for topical use.