Increased Interleukin-19 Expression in Cutaneous T-cell Lymphoma and Atopic Dermatitis

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Interleukin-19 (IL-19), a pro-inflammatory cytokine known to stimulate the production of T helper type 2 (Th2) cytokines, is induced by IL-17A and highly expressed in the lesional skin of psoriasis and atopic dermatitis (AD). This aim of this study was to investigate whether IL-19 is involved in cutaneous T-cell lymphoma (CTCL) and AD. IL-19 levels were significantly higher in the sera of patients with AD and those with advanced-stage CTCL than in normal controls, correlating significantly with clinical disease markers. IL-19 mRNA levels in lesional skin of both diseases were significantly elevated. Immunohistochemical staining revealed that IL-19 was expressed in the epidermis of AD skin and CTCL skin. In vitro, IL-17A and IL-4 increased IL-19 mRNA expression in human keratinocytes. Thus, IL-19 was increased in the sera and skin of AD and CTCL. These results suggest that IL-19 is important for bridging Th17 to Th2 in these diseases.

Key words: IL-19; atopic dermatitis; cutaneous T-cell lymphoma; keratinocytes; IL-4; IL-17A.

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Materials and Methods

Tissue and serum samples
Messenger RNA (mRNA) was obtained from biopsy materials of lesional skin of MF/SS (n = 17; patch MF 4, plaque MF 3, tumour MF 5, erythrodermic MF 2, SS 3; mean ± standard deviation (SD) age: 57.6 ± 12.7 years; 12 males and 5 females), AD (n = 7; mean ± SD age: 36.3 ± 16.0 years; 5 males and 2 females), and normal skin adjacent to benign skin tumours (n = 8; mean ± SD age: 49.1 ± 21.0 years; 4 males and 4 females) using RNeasy Fibrous Tissue Mini Kit (QIAGEN, Valencia, CA, USA). Samples for immunohistochemistry were lesional skin of MF/SS (n = 10; patch MF 2, plaque MF 3, tumour MF 3, SS 2) or AD (n = 10), and normal skin adjacent to benign skin tumours (n = 10). Serum samples were obtained from 28 patients with MF/SS (patch MF 7, plaque MF 10, tumour MF 9, erythrodermic MF 1, SS 1; mean ± SD age: 54.5 ± 18.1 years; 15 males and 13 females), 35 patients with AD (10 mild cases, 10 moderate cases, and 15 severe cases; mean ± SD age: 30.8 ± 12.0 years; 23 males and 12 females), and 17 healthy control subjects (mean ± SD age: 47.6 ± 20.6 years; 11 males and 6 females). The healthy controls had no history of allergy, AD, psoriasis, or CTCL. All samples were collected during daily clinical practice in the University of Tokyo Hospital.

The medical ethics committee of the University of Tokyo approved...
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RESULTS

Increased IL-19 protein levels in sera of patients with AD and advanced stage cutaneous T-cell lymphoma

To investigate IL-19 involvement in AD and CTCL, we first examined IL-19 protein levels in sera of patients with AD and CTCL. IL-19 protein levels in sera of AD patients were significantly elevated compared with those of normal controls (Fig. 1A). Serum IL-19 levels increased according to AD severity (Fig. 1B). IL-19 protein levels in sera of patients with CTCL tended to be increased, although there was no statistical significance (Fig. 1A). When divided by clinical stages, patients with advanced stage CTCL showed significant elevation in serum IL-19 levels compared with normal controls and patients with early stage CTCL (Fig. 1C). Thus, serum IL-19 levels were elevated in AD and patients with advanced stage CTCL.

Factors correlating with serum IL-19 levels

The association between serum IL-19 levels and clinical disease markers was examined. Eczema Area and Severity Index (EASI) score, a severity index of AD, was positively associated with serum IL-19 levels in patients with AD (Fig. 2A). Moreover, serum levels of thymus and activation-regulated chemokine (TARC) and the number of eosinophils in peripheral blood significantly correlated with serum IL-19 levels in patients with AD (Fig. 2B, C). Serum IL-19 levels were positively associated with serum soluble IL-2 receptor (sIL-2R) levels in patients with CTCL (Fig. 2D). Thus, serum IL-19 levels positively correlated with clinical disease markers of AD and CTCL.

Increased IL-19 mRNA expression levels in lesional skin of AD and CTCL

Levels of expression of IL-19, IL-4 and IL-17A mRNA in normal skin and lesional skin of AD and CTCL were examined. IL-19 mRNA levels were significantly elevated in both AD and CTCL skin compared with those of normal skin (Fig. 3A). In AD skin, IL-19 mRNA expression levels were significantly higher than those in normal skin. In CTCL skin, IL-19 mRNA expression levels were significantly higher than those in normal skin. The measured values from individual patients were plotted by dots. Error bars represent standard errors of the mean (SEM). *p < 0.05. (B) Correlation between IL-19 and IL-4 mRNA levels in AD lesional skin (n = 7). (C) Correlation between IL-19 and IL-4 in CTCL lesional skin (n = 17). (D) Correlation between IL-19 and IL-17A in CTCL lesional skin (n = 17). (E) IL-19 relative mRNA levels in human epidermal keratinocytes stimulated with IL-17A (1, 10, 100 ng/ml) or medium control for 24 h. The mean level in medium control was arbitrarily set to 1 and all values represent the mean±SEM (n = 6). All experiments were done twice and representative results are shown. *Indicates significant difference compared with medium control. *p < 0.05 by Mann–Whitney U test. (F) IL-19 relative mRNA levels in human epidermal keratinocytes stimulated with IL-4 (10, 100 ng/ml) or medium control for 24 h. The mean level in medium control was arbitrarily set to 1 and all values represent the mean±SEM (n = 6). All experiments were done twice and representative results are shown. *Indicates significant difference compared with medium control. *p < 0.05.
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**IL-19 expression was increased in NHEK by IL-17A or IL-4 stimulation**

To study regulation of IL-19 expression by keratinocytes, IL-19 mRNA levels were investigated with quantitative RT-PCR using NHEK stimulated with IL-17A, IL-4, IL-13, IL-22, IFN-γ, or medium only. IL-17A increased IL-19 mRNA expression by NHEK in a dose-dependent manner (Fig. 3E). Stimulation with IL-4 also significantly increased IL-19 mRNA levels, although the levels of increase were much smaller than IL-17A stimulation (Fig. 3F). Stimulation with IL-13, IL-22, or IFN-γ did not change IL-19 mRNA expression (data not shown). Thus, IL-19 mRNA expression by human keratinocytes increased by IL-17A or IL-4 stimulation, which was consistent with previous reports (4, 5, 7, 8).

**Immunohistochemical staining of IL-19 in lesional skin of AD, CTCL and psoriasis**

Immunohistochemical staining of IL-19 revealed that epidermis in AD skin strongly expressed IL-19 (Fig. 4). Keratinocytes in CTCL lesional skin were slightly positive for IL-19 (Fig. 4). Lesional skin of psoriasis was also stained positive, as reported previously (16, 23). The immunohistochemistry of 10 cases in each group are summarized in Table I. Thus, epidermis of AD, CTCL and psoriasis lesional skin expressed IL-19.

**DISCUSSION**

This study showed high levels of IL-19 expression in the sera and lesional skin of patients with AD and CTCL. In patients with AD and CTCL, serum IL-19 levels positively correlated with clinical disease markers. In lesional skin, IL-19 mRNA levels positively correlated with IL-4 mRNA levels in AD, and with IL-4 and IL-17A mRNA levels in CTCL. In vitro, IL-17A and IL-4 increased IL-19 expression by human keratinocytes. Immunohistochemical staining revealed that epidermis in AD, CTCL, and psoriasis lesion skin expressed IL-19. Taken together, IL-19 is highly expressed in CTCL as well as AD, suggesting a possible role in the development of these diseases.

IL-19 expression was increased at both mRNA and protein levels in AD lesional skin (see Figs 3A and 4), which was consistent with previous reports (4). CTCL lesional skin, whose cytokine/chemokine profiles are similar to AD skin (24), also expressed IL-19 (Figs. 3A and 4). Moreover, we first showed serum IL-19 levels were significantly increased in both diseases, positively correlating with the clinical severity (Fig. 1) and other disease markers (Fig. 2). EASI, serum TARC levels, and the number of eosinophils clearly indicate AD severity (25–27) and serum sIL-2R levels reflect CTCL disease activity (28). The role of IL-19 has been proposed in psoriasis, but our results suggest that IL-19 is also involved in AD and CTCL.

IL-19 mRNA levels positively correlated with IL-4 mRNA levels in AD and CTCL skin (Figs. 3B and C). Previous studies reported that IL-19 induced Th2 cytokines (3, 18) and that IL-19 expression was up-regulated by Th2 cytokines (5, 29). Serum IL-19 levels were up-regulated in patients with asthma, a representative Th2 disease (18). Our results are consistent with the fact that AD and CTCL at advanced stage are both Th2-dominant diseases.

Both IL-4 and IL-17A have been reported to up-regulate IL-19 expression by keratinocytes (4, 5, 8). In this study, stimulation with IL-17A significantly enhanced IL-19 mRNA expression by human keratinocytes in a

| Table I. Interleukin-19 expression in lesional skin of normal skin, atopic dermatitis (AD), cutaneous T-cell lymphoma (CTCL) and psoriasis |
|-----------------|-----------------|-----------------|-----------------|
|                 | Negative | Weak | Positive | Strong positive |
| Normal          | 8        | 2    | 0        | 0               |
| AD              | 1        | 2    | 1        | 6               |
| CTCL            | 1        | 5    | 2        | 2               |
| Psoriasis       | 1        | 4    | 5        | 0               |

Fig. 4. Immunohistochemistry of interleukin-19 (IL-19) in normal skin and lesional skin of atopic dermatitis (AD), cutaneous T-cell lymphoma (CTCL) or psoriasis.
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The authors declare no conflicts of interest.

REFERENCES


