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Decreased IL-15 May Contribute to Elevated IgE and Acute Inflammation in Atopic Dermatitis

Peck Y. Ong,* Qutayba A. Hamid,‡ Jeffrey B. Travers,‡ Ian Strickland,* Muhamed Al Kerithy,† Mark Boguniewicz,*§ and Donald Y. M. Leung2§§

PBMC and acute skin lesions of patients with atopic dermatitis (AD) are characterized by increased IL-4 and IL-13, but decreased IFN-γ production. This bias toward an increased Th2 cytokine profile may contribute to the elevated IgE levels and acute skin inflammation seen in AD. In this study, we examined the levels of IL-15, a Th1-like cytokine, in the PBMC and the skin lesions of AD patients. IL-15 secretion by Staphylococcal enterotoxin B-treated PBMC of AD patients was significantly lower than that of normals and psoriasis patients (p < 0.001). Membrane-bound IL-15 expression as measured by mean fluorescence intensity and percentage of IL-15-positive cells in Staphylococcal enterotoxin B-treated monocytes of AD patients (644 ± 49% and 12.7 ± 0.6%, respectively) were significantly lower than that of normals (869 ± 56% and 15.8 ± 1.2%, respectively) and psoriasis patients (1488 ± 217% and 22.7 ± 0.8%, respectively; p < 0.0007 and p < 0.0001, respectively). The membrane-bound IL-15 expression was also significantly lower in the control monocytes of AD patients compared with that in normals and psoriasis patients. There was no significant difference in the absolute number or percentage of monocytes between the study subjects. However, psoriasis skin lesions were found to have significantly more IL-15 mRNA-expressing cells (22.4 ± 1.7) compared with that in acute AD (7.5 ± 1.7) and chronic AD (13.7 ± 1.7) skin lesions (p < 0.05). IL-15 enhanced IFN-γ production by the PBMC of AD patients (p < 0.01), but not by that of normal individuals or psoriasis patients. In addition, IL-15 was found to suppress IgE synthesis (p < 0.01) by the PBMC of AD patients. These data support the concept that reduced IL-15 expression may contribute to the pathogenesis of AD. The Journal of Immunology, 2002, 168: 505–510.

A topic dermatitis (AD) is a chronic allergic skin disease that is characterized by an increased production of Th2 cytokines (e.g. IL-4 and IL-13) and IgE (1), but a decreased production of the Th1 cytokine, IFN-γ (2) in the peripheral blood and acute skin lesions. Th2 cytokines are thought to play a role in the pathogenesis of AD by enhancing IgE synthesis, eosinophilia, and induction of adhesion molecules that are involved in the migration of inflammatory cells into the skin lesions (3, 4). A decrease in IFN-γ, in contrast, contributes to the increased production of Th2 cytokines and IgE in AD because this cytokine has been shown to suppress the proliferation of Th2 cells (5) and IgE synthesis (6).

IL-15 is a cytokine with widespread mRNA expression in various tissues, including placenta, lung, and primarily monocytes in the peripheral blood (7, 8). It stimulates the proliferation of T cells (9) and the production of IgM, IgG1, and IgA, but not IgE, by B cells (10). IL-15 is generally thought to be a Th1 cytokine (reviewed in Ref. 11). The capability of IL-15 in inducing IFN-γ expression has been well documented (12, 13). In healthy individuals, IL-15 has been shown to favor a Th1 response by increasing the expression of IFN-γ (14). Therefore, we were interested in determining whether IL-15 production is decreased in AD, and its expression in atopic skin lesions.

In this study, we examined the secretion of IL-15 and expression of membrane-bound IL-15 by the PBMC of AD patients. We next compared the expression of IL-15 mRNA in the skin lesions of AD to that of psoriasis, a Th1-mediated skin disease (15). To determine the functional role of IL-15 on the AD immune response, we also examined its effects on the modulation of IFN-γ production and IgE secretion by PBMC of AD patients.

Materials and Methods

Patients, control subjects, and skin biopsy specimens

Nine patients with moderate to severe AD (skin involvement of 15–80%) and elevated total serum IgE (623–5760 kU/L) were studied. Their diagnosis was based on the criteria of Hanifin and Rajka (16). None of the patients had received any systemic corticosteroids previously. All patients were off topical corticosteroids for 1 wk before their blood donation and skin biopsy. Informed consent was obtained from all subjects before the study. Two control groups were studied: 10 healthy subjects with no history of AD, allergic rhinitis, or asthma (total serum IgE <100 kU/L) and six patients with psoriasis (skin involvement of 10–50% and total serum IgE <50 kU/L). Patients with psoriasis were not on any systemic treatment, and topical corticosteroids were withheld for 1 wk before blood donation or skin biopsy.

Five AD and five psoriasis patients gave consent for skin biopsy. A total of 10 3-mm punch skin biopsy specimens were obtained from these AD patients: five specimens from acute erythematous AD lesions of less than 3 days’ onset, and five specimens from chronic lichenified AD lesions of
performed by omission of the primary Ab or the use of irrelevant isotype-munoreactive cells were stained reddish-brown. Negative controls were salmon sperm DNA) overnight at 4°C in a humid chamber. The sections had a detection limit of 100 ng/ml of Staphylococcal enterotoxin B (SEB; Toxin Technology, Santa Monica, CA) was used together with anti-CD68 mAb in double negative controls were performed using nonspecific mouse Ig. For color development, a nitroblue tetrazolium and tetrazolium blue solution (Tissue-Tek; Miles Inc., Elkhart, Indiana), and stored at −80°C until used. Sense and anti-sense digoxigenin (Dig)-labeled riboprobes were prepared from cDNA encoding for IL-15 (a kind gift from Immunex, Seattle, WA). The cDNA was inserted into a pGEM vector, linearized, and transcribed in the presence of Dig-labeled uridine triphosphate. Cryostat sections were then permeabilized and incubated with Dig-labeled IL-15 probe at a concentration of 300 ng/ml in hybridization buffer (50% formamide, 5× Denhardt’s solution, 5× SSC, and 500 µg/ml denatured salmon sperm DNA) overnight at 40°C in a humid chamber. The sections were then washed extensively with SSC and treated with RNase A to remove unhybridized RNA and then incubated with 1/500–1/5000 dilutions of Dig-alkaline-phosphatase conjugate in a humid box overnight at room temperature. Color development was achieved with nitroblue tetrazolium salt in equalization buffer. The sections were then counterstained with hematoxylin and examined under a microscope. Results were expressed as number of positive cells per high-power field.

**Immunohistochemistry and double immunohistochemistry studies**

Immunohistochemistry of skin biopsies from AD patients and controls was performed using the alkaline phosphatase-anti-alkaline phosphatase method as previously described (18). Mouse anti-CD68 mAb (DAKO, Carpinteria, CA) was used to detect macrophages in the skin sections, and negative controls were performed using nonspecific mouse Ig. For colo- 

**PBMC culture and cytokine measurements**

PBMC from AD patients and controls were isolated from heparinized venous blood by density gradient centrifugation on Ficoll-Paque (Pharmacia Biotech, Uppsala, Sweden), washed three times in HBSS (Mediatech, Herndon, VA), and resuspended at 2 × 10^6 cells/ml in RPMI 1640 with 10% (v/v) heat-inactivated FCS supplemented with 2 mM l-glutamine, 50 µg/ml streptomycin, and 100 U/ml penicillin. For cytokine measurements, 2 × 10^6 PBMC/ml culture medium were incubated in the presence of 100 ng/ml of Staphylococcal enterotoxin B (SEB; Toxin Technology, Sarasota, FL) for 96 h and supernatants were collected and stored at −80°C until assayed. Commercial ELISA kits were used to determine the levels of IL-15 (R&D Systems), and IFN-γ (Endogen, Woburn, MA). The IL-15 ELISA had a detection limit of <1 pg/ml (20), whereas the IFN-γ ELISA had a detection limit <2 pg/ml.

**Membrane-bound IL-15 staining and flow cytometric analysis**

Staining of membrane-bound IL-15 was performed using a modified method described by Musso et al. (21). Briefly, PBMC were washed once with a wash buffer (PBS with 2% (v/v) FCS and 0.02% (w/v) sodium azide (Sigma, St. Louis, MO) and resuspended at 8 × 10^6 cells/ml in a staining solution (PBS with 5% (v/v) FCS, 1% (w/v) human Ig (Bayer, Elkhart, IN) and 0.02% sodium azide) in a 96-well microtiter plate. The cells were incubated with the anti-IL-15 mAb or an isotype-matched mAb for 45 min at 4°C. The cells were then washed twice with the wash buffer and resuspended in staining solution and incubated with a goat anti-mouse FITC conjugate for 30 min at 4°C. The cells were then washed four times with the wash solution and resuspended in staining solution and incubated with anti-CD64 PE for another 30 min at 4°C. Finally, the cells were washed three times and fixed in 200 µl of 1% (v/v) formaldehyde (Poly-sciences, Warrington, PA) in PBS. Analysis was performed using a FACS-Calibur flow cytometer (BD Biosciences, Mountain View, CA). List mode multiparameter data files (each file with forward scatter, side scatter, and the two fluorescence parameters, FITC and PE) were analyzed using the CellQuest MacIntosh program (BD Biosciences). Analyses were performed using a light scatter gate including only viable cells and a gate based on expression of CD64^+ monocytes. Isotype-matched mAb control was used to verify the staining specificity of IL-15.

**Measurement of Ig synthesis**

For determination of IgE and IgG, 2 × 10^6 PBMC/ml were incubated with 400 U/ml rIL-4 and 1 µg/ml anti-CD40 mAb and in the presence or absence of 10 ng/ml rIL-15 for 14 days. The supernatants were then harvested and stored at −80°C until assayed. In selected experiments, the effect of anti-IFN-γ on IgE synthesis was studied by adding 10 µg/ml anti-IFN, an amount capable of neutralizing 5000 U of IFN-γ.

The IgE and IgG measurements were conducted using commercial ELISA kits (Bethyl Laboratories, Montgomery, TX) according to the manufacturer’s instructions. Briefly, 96-well microtiter plates (Nunc Maxisorp; Cole-Parmer, Vernon Hills, IL) were coated with 0.1 ml of an affinity-purified polyclonal goat anti-human IgE (Bethyl Laboratories) diluted 1/100 in 0.05 M NaHCO_3 at pH 9.6 (1/100, v/v) for 1 h at room temperature. The wells were washed three times each with 0.2 ml of 0.05% Tween 20 (Sigma-Aldrich) in 50 mM TBS blocked with 1% (w/v) BSA (Sigma-Aldrich) in 50 mM TBS, pH 8.0, for 30 min at room temperature. The wells were washed three times and 0.1 ml of a 1/12,500 dilution of a HRP-conjugated goat anti-human IgE (Be- 

thyl Laboratories) in 50 mM TBS, pH 8.0, with 1% (w/v) BSA and 0.05% Tween 20 for 1 h at room temperature. The wells were then washed three times and incubated with 0.1 ml of a 1/12,500 dilution of a HRP-conjugated goat anti-human IgG (Be- 

thyl Laboratories) in 50 mM TBS, pH 8.0, with 1% (w/v) BSA and 0.05% Tween 20 for 1 h at room temperature. The wells were then washed five times and developed with 0.1 ml 1:1 mixture of 3,3,5,5'-tetramethylben-

zidine substrate/hydrogen peroxide solution (Kirkegaard & Perry Labora-

tories, Gaithersburg, MD) at room temperature for 20 min. The color de-

t 

veloped was stopped with 0.1 ml of 1 M H_2SO_4 and the OD was read at 450 nm. The concentration of IgE in the supernatants was read from an IgE standard curve. The lower limit of sensitivity of this assay was 1.87 ng/ml.

The procedure for IgG ELISA was identical with that for IgE except for the initial capture Ab (an affinity-purified polyclonal goat anti-human IgG Ab), the detection Ab (a HRP-conjugated goat anti-human IgG Ab), and the IgG standards, which were obtained from Bethyl Laboratories. The lower limit of sensitivity of this assay was 15.6 ng/ml.
psoriasis patients. MFI for membrane-bound IL-15 was significantly lower in control monocytes from AD patients than monocytes from normal individuals and psoriasis patients, with the values 398 ± 11006, 42, 569 ± 11006, and 909 ± 100, respectively (p < 0.0001 by ANOVA, Fig. 3). Statistically significant differences in membrane-bound IL-15 MFI of the SEB-stimulated monocytes were also noted between AD patients, normal individuals, and psoriasis patients (644 ± 49 vs 869 ± 56 vs 1488 ± 217, p < 0.0007 by ANOVA; Fig. 3).

In the analysis of the percentage of cells positive for membrane-bound IL-15 in the control monocytes, significant differences were noted between AD patients, normal individuals, and psoriasis patients, with values of 6.1 ± 0.9, 10.9 ± 1.1, and 13.1 ± 1.1%, respectively (p < 0.0007 by ANOVA; Fig. 4). Statistically significant differences were also noted in the percentage of SEB-stimulated monocytes positive for membrane-bound IL-15 between AD patients, normal individuals, and psoriasis patients (12.7 ± 0.6 vs 15.8 ± 1.2 vs 22.7 ± 0.8, p < 0.0001 by ANOVA; Fig. 4). There was no significant difference (p > 0.05) in the absolute number of monocytes or percentage of monocytes per total viable cells between AD patients and normal individuals or psoriasis patients (Table I). Therefore, the differences in membrane-bound IL-15 levels between AD patients and normal individuals or psoriasis patients are not due to differences in the number or percentage of monocytes between these study subjects.

FIGURE 1. Comparison of IL-15 secretion by SEB-stimulated PBMC from nine AD patients, 10 normal individuals, and six psoriasis patients. PBMC were incubated with 100 ng/ml SEB and supernatants were measured for IL-15 by ELISA. An asterisk indicates that by using Tukey-Kramer HSD to compare each group, a significant difference was noted between AD and normal individuals (p < 0.05) or psoriasis patients (p < 0.05). Results from each individual and mean values are shown.

FIGURE 2. Representative histograms showing MFI analysis of membrane-bound IL-15 in control (left) and SEB-stimulated (right) monocytes of AD patients vs normal individuals and psoriasis patients. PBMC were cultured in medium (control) or in the presence of 100 ng/ml SEB for 96 h. The cells were then analyzed by flow cytometry for the expression of membrane-bound IL-15 on CD64+ monocytes. IL-15 fluorescence is represented by dark histograms and the background staining of an isotype-matched control mAb is represented by lighter histograms.

FIGURE 3. Comparison of MFI for membrane-bound IL-15 in control or SEB-treated monocytes between six AD patients, six normal individuals, and five psoriasis patients. An asterisk indicates that by using Tukey-Kramer HSD to compare each group, a significant difference was noted between AD patients and psoriasis patients (p < 0.05) in both control and SEB-stimulated monocytes. Results from each individual and mean values are shown.
Acute AD skin lesions have significantly fewer IL-15 mRNA⁺ cells than chronic AD and psoriasis skin lesions

We next examined IL-15 mRNA expression in acute vs chronic AD skin lesions and compared their values to psoriatic skin lesions. Statistically significant differences were found in the number of IL-15 mRNA-expressing cells between acute AD vs chronic AD or psoriasis skin lesions. A pairwise comparison revealed a significantly higher number of IL-15 mRNA-expressing cells in psoriasis skin lesions compared with acute or chronic AD skin lesions (p < 0.05, Tukey-Kramer honestly significant difference (HSD)). Co-localization studies revealed that 25 ± 4.2% of IL-15-positive cells were CD68⁺ macrophages. Unlike PBMC where IL-15 was exclusively expressed in monocytes/macrophages, in the skin, IL-15 mRNA also co-localized to keratinocytes, particularly basal cells, as well as infiltrating T cells and dermal fibroblasts.

These differences in IL-15 expression could not be accounted for by variation in monocyte-macrophage numbers between AD vs psoriasis skin lesions, as immunohistochemical studies revealed similar numbers of CD68-positive monocyte-macrophages among the three subject groups with mean number ± SEM of CD68 cells per high-power field in acute AD of 12.2 ± 3.7; in chronic AD of 11.0 ± 2.9; and in psoriasis of 10.9 ± 3.2.

Functional effects of IL-15 significantly on AD cells

To determine the potential role of IL-15 on immune responses by PBMC from AD patients, we examined the effects of IL-15 on IFN-γ and Ig synthesis. In Fig. 6, SEB-stimulated PBMC from six AD patients were incubated with rIL-4 and anti-CD40 mAb to promote IgE synthesis, in the absence or presence of rIL-15. Fig. 7 shows that IL-15 significantly suppresses IgE synthesis (p < 0.01), but had no effect on IgG synthesis (p = 0.2). Anti-IFN-γ at 10 μg/ml, which is capable of neutralizing 5000 U of IFN-γ, did not reverse the suppression of IgE synthesis by IL-15 (data not shown).

Discussion

An important feature of AD is a decrease in IFN-γ expression by the systemic immune system and in acute skin lesions (2). This decrease in IFN-γ may contribute to the increased Th2 cytokine profile and IgE production seen in AD because IFN-γ has been shown to suppress the proliferation of Th2 cells (5) and IgE (6).

Table 1. Absolute and relative number of monocytes in PBMC of AD, normal, and psoriasis controls

<table>
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<th>AD</th>
<th>Normal</th>
<th>Psoriasis</th>
<th>p*</th>
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<tbody>
<tr>
<td>Absolute no.</td>
<td>12,315 ± 904</td>
<td>11,004 ± 904</td>
<td>11,937 ± 900</td>
<td>NS</td>
</tr>
<tr>
<td>% Monocyte</td>
<td>17 ± 2</td>
<td>16 ± 2</td>
<td>19 ± 2</td>
<td>NS</td>
</tr>
</tbody>
</table>

* Pairwise comparison using Tukey-Kramer HSD shows no difference in the absolute number of monocytes and percentage of monocytes per total viable cells between AD patients and normal individuals or psoriasis patients.
Th2 cytokines, in addition to inducing IgE synthesis, may be crucial in the initiation of the acute inflammation of AD by recruiting inflammatory cells into the acute skin lesions (4). The pathogenic role of IgE in AD includes its capability in facilitating the capture and presentation of allergen by Langerhans cells to skin T cells (22).

The decreased secretion of IL-15 by PBMC of AD patients shown in this study provides evidence that dysregulation of IL-15 may play a role in the pathogenesis of AD. This decrease in IL-15 secretion was further supported by a decrease in membrane-bound IL-15 expression by the monocytes of AD patients and a decreased expression of IL-15 mRNA in the acute and chronic AD skin lesions compared with that in psoriasis skin lesions. The increased expression of IL-15 mRNA in psoriasis skin lesions is consistent with the role of IL-15 as a Th1 cytokine because Th1 cells, but not Th2 cells, have been found to infiltrate psoriasis skin lesions (15). High expression of IL-15 in psoriatic plaques has also been reported in another study (23). The decreased expression of IL-15 mRNA in acute AD skin lesions compared with that in chronic AD skin lesions is also consistent with the biphasic model for the pathogenesis of AD (24). In this model, the acute phase of AD is predominated by a Th2-like response, whereas the chronic phase of AD is predominated by a Th1-like response. The biphasic model has also been demonstrated by analysis of T cell cytokine profile during the development of atopy patch test lesions (25, 26). Increased expression of IL-4 in the skin lesions was observed during the first 24 h after allergen application (acute phase) and then declined after that. In contrast, increased expression of IFN-γ in the skin lesions was not observed until after 48 h (chronic phase). This model is also supported by skin biopsy studies from AD patients in which acute AD skin lesions were found to have increased expression of IL-4 (27) and IL-13 (28) mRNA, whereas chronic AD skin lesions were found to have increased expression of IFN-γ mRNA (29).

The exact mechanisms by which IL-15 may contribute to the pathogenesis of AD remain to be elucidated. Our data indicate that IL-15 is capable of enhancing IFN-γ production by the PBMC of AD patients. It is possible that increased expression of IL-15 in chronic AD skin lesions also contributes to the observed IFN-γ expression in these skin lesions. A recent study in a murine model of asthma indicated that IL-15 may suppress allergic inflammation by enhancing the production of IFN-γ by CD8⁺ T cells (30). This is of interest because IFN-producing CD8⁺ T cells may play a crucial role in the development and maintenance of chronic atopic dermatitis (31). A recent study suggests that IL-15 may act as an anti-apoptotic cytokine in the maintenance of chronic AD (32). The role of IL-15 in sustaining chronic skin inflammation has been supported by the studies of IL-15 expression in keratinocytes of psoriasis lesions (23) and dermal fibroblast (33). In contrast, the decrease of systemic IL-15 in AD may account for the decreased NK cell activity associated with this skin disease (34) because IL-15 plays a pivotal role in the development, survival, and function of NK cells (35).

In contrast to its enhancing effects on IFN-γ production, we found that IL-15 suppressed IgE synthesis. Therefore, a decrease in IL-15 may contribute to the elevation of IgE levels in AD. However, the suppression of IgE synthesis by IL-15 was not reversible by anti-IFN-γ, indicating that IL-15 may have a role in the pathogenesis of AD that is independent of IFN-γ.

In summary, our current study shows that IL-15 is decreased in the PBMC and acute skin lesions of AD patients. This decrease may contribute to the inflammation of acute AD lesions and the elevated IgE levels seen in AD patients. However, increased IL-15 may play a role in the sustained inflammation of chronic AD and psoriasis lesions. These findings suggest that modulation of IL-15 levels in AD may provide a novel intervention in the treatment of this disease in the future.

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References

IL-15 IN ATOPIC DERMATITIS


