Expression levels of transcription factor PU.1 and interleukin-9 in atopic dermatitis and their relation to disease severity and eruption types

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Abstract
Background The role of immunological factors in atopic dermatitis (AD) pathogenesis is well established. T-helper (TH) cells are central in AD pathogenesis. A relatively new subset of T cells, Th9 cells, was shown to be involved in the development of allergic asthma and allergic rhinitis, while its role in AD is still to be investigated. This study aimed to measure gene expression levels of interleukin-9 (IL-9) and PU.1, and to examine relationships with disease severity, serum IgE, and eruption types in AD patients.

Methods The study enrolled 30 AD patients, 30 psoriasis patients, and 30 healthy subjects. The severity of AD was assessed using the SCORAD index. IL-9 and PU.1 expressions were measured by using real-time quantitative polymerase chain reaction (RQ-PCR). Serum IgE was measured by IgE (human) enzyme-linked immunosorbent assay (ELISA) Kit.

Results IL-9 and PU.1 gene expressions were significantly higher in AD patients than in controls ($P_1 = 0.007$, $P_2 < 0.001$, respectively). In the atopic dermatitis patients, expression of IL-9 and PU.1 were significantly positively correlated with SCORAD index ($P_1 = 0.004$, $P_2 = 0.002$) and clinically with erythema and edema scores. IL-9 and PU.1 expressions were positively significantly correlated ($P = 0.005$) and positively correlated with serum IgE in the AD group ($P_1 = 0.017$, $P_2 = 0.023$). No significant difference was noted between AD patients with or without histories of other atopies regarding expression levels of IL-9 and PU.1 ($P_1 = 0.677$, $P_2 = 0.135$).

Conclusions PU.1 and IL-9 may play a role in AD pathogenesis and relate to disease severity and clinical eruption types.
Subjects and methods

Subjects
This study was conducted on 30 atopic dermatitis patients (group A) diagnosed according to the criteria of Hanifin and Rajka.\textsuperscript{17} While AD is considered a polar Th2 disease in the acute phase with a partial shift to Th1 during the chronic phase,\textsuperscript{18} psoriasis is considered a model Th1 disease.\textsuperscript{19} Therefore, we compared expression levels of PU.1 and IL-9 in AD and psoriasis, the well-known opposite poles of the Th1 versus Th2 paradigm. Control subjects included 30 age- and gender-matched psoriatic patients not thus far diagnosed as having any atopic disorder (group B) and another 30 age- and gender-matched healthy control subjects without atopic disorders or inflammatory skin diseases (group C). Patients and controls were selected from the dermatology outpatient clinic of the Alexandria Main University Hospital, Egypt. An informed consent was obtained from all study subjects or their guardians, in cases of children. The study protocol was approved by the Ethics Committee of the Faculty of Medicine, Alexandria University, and followed the International Ethical Guidelines of the 1975 Declaration of Helsinki.

Exclusion criteria
Exclusion criteria included treatment with systemic glucocorticoids or other immunosuppressive agents within the previous 6 months as well as local treatment with a glucocorticoid or calcineurin inhibitor within the previous week.

Methods
The atopic dermatitis patient group was subjected to history taking, general medical examination, and a local dermatologic examination for assessment of the Severity Scoring of Atopic Dermatitis (SCORAD) index.\textsuperscript{20} Skin lesion severity (erythema score, edema/papule score, oozing/crust score, excoriation score, lichenification score, and xerosis score) was also evaluated in the most affected areas in the patients with AD.

Three milliliters of venous blood was withdrawn from all patients and controls under total aseptic technique in BD Vacutainer\textsuperscript{®} blood collection tubes containing K\textsubscript{2}EDTA (di-potassium ethylenediamine tetra-acetic acid). The samples were then subjected to the following.

I. Measurement of PU.1 and IL-9 gene expression levels
Total RNA was isolated from peripheral blood mononuclear cells (PBMCs) using QIAamp RNA blood mini kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. The concentration and purity of RNA were measured at 260, 280, and 230 nm using NanoDrop2000 Spectrophotometer (Thermo Scientific, Wilmington, DE, USA). A260:A230 ratio greater than 1.7 and A260:A280 ratio greater than 2.0 indicated highly pure RNA. A reverse transcriptase kit (QuantiTect Reverse Transcription Kit, Qiagen) was used for complementary DNA (cDNA) synthesis. The expression of PU.1 and IL-9 mRNA was analyzed with the QuantiFast probe assays (Cat.no. 243132, Qiagen) and GAPDH was used as an endogenous reference. Real-time quantitative polymerase chain reaction (RQ-PCR) was performed on a Rotor-Gene Q (Qiagen).\textsuperscript{13} Expression levels were determined using the 2\textsuperscript{ΔΔCt} method. The 2\textsuperscript{ΔΔCt} method is the method of relative quantification that is most frequently found in popular software packages to quantify gene expression levels. The 2\textsuperscript{ΔΔCt} method is easy to perform, and it can be valid under optimal conditions.\textsuperscript{21}

II. Serum IgE level measurement
Serum IgE level was measured by IgE (Human) enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, Bio-Techne, Minneapolis, USA).

Statistical analysis of the data
Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. Qualitative data were described using number and percent. Quantitative data were described using range (minimum and maximum); mean, standard deviation, and median. Significance of the obtained results was judged at the 5% level. The used tests were Chi-square test for categorical variables, to compare between different groups, Kruskal–Wallis test for abnormally quantitative variables, to compare between more than two studied groups, Mann–Whitney test for abnormally quantitative variables, to compare between two studied groups, and Spearman coefficient to correlate between two abnormally quantitative variables.

Results
The mean ages ± SD for the study subjects were 13.33 ± 10.29 years (2.0–36.0 years), 13.45 ± 5.85 years (6.50–22.0 years), and 50 ± 7.45 years (6.0–30.0 years) for the atopic dermatitis, psoriasis, and healthy control groups, respectively. Males constituted 53.3%\textsuperscript{16}, 46.7%\textsuperscript{14}, and 40.0%\textsuperscript{12} whereas females were 46.7%\textsuperscript{14}, 53.3%\textsuperscript{16}, and 60.0%\textsuperscript{18} for the atopic dermatitis, psoriasis, and healthy control groups, respectively. All three groups were age- and gender-matched.

The reported number of flares/year experienced in the preceding year ranged from 1.0–7.0/year with a mean of 3.17 ± 2.28/year. Calculated SCORAD index ranged from 8.50–49.80 with a mean of 25.42 ± 13.37. Out of the 30 atopic dermatitis patients, 23 subjects (76.7%) confirmed a positive personal history of other atopic disorders whereas the remaining seven subjects (23.3%) had negative personal history of other atopic disorders.

Expression levels of interleukin-9 (IL-9) and PU.1 and serum IgE level
IL-9 and PU.1 expression levels and the serum IgE were highest among the AD group followed by the psoriasis group and...
were the least in the healthy control group. Differences in the IL-9 and PU.1 expression levels and serum IgE in the three studied groups were statistically significant. (P1 = 0.007, P2 < 0.001, P3 < 0.001, respectively) (Table 1).

**Relationship of the expression levels of IL-9 and PU.1 and SCORAD**

There was a significant positive correlation between the expression levels of both IL-9 and PU.1 and the SCORAD index. (P1 = 0.004, P2 = 0.00, respectively) (Table 2).

**Relationship of interleukin-9 (IL-9) and PU.1 expression levels to serum IgE in patients with AD**

In the AD group, both IL-9 and PU.1 expression levels significantly positively correlated with serum IgE levels (P = 0.017 and P = 0.023, respectively). Also, within the AD group, there was a significant positive correlation between the expression levels of IL-9 and PU.1 (P = 0.005). In the psoriasis control group, only IL-9 and PU.1 expressions were significantly correlated (P = 0.001). In the healthy control group, none of the three variables were correlated (Table 3).

**Relationship of the expression levels of interleukin-9 (IL-9), PU.1, serum IgE level, and history of other atopies in patients with AD**

The history of other atopic disorders did not correlate with the expression levels of IL-9, PU.1, or serum IgE among the AD patients. No statistically significant differences between patients with or without a history of other atopies regarding the expression levels of IL-9, PU.1 and serum IgE were detected. (P1 = 0.677, P2 = 0.135, P3 = 0.492, respectively) (Table 4).

**Relationship of IL-9 and PU.1 expression levels with eruption type and itch score in patients with AD**

Among the six types of eruptions (erythema, edema/papule, oozing/crust, excoriation, lichenification, and xerosis), IL-9 expression was significantly correlated only with the erythema score (r = 0.337, P = 0.034) and the edema/papule score (r = 0.311, P = 0.047). No correlation was detected between IL-9 expression and the excoriation score (r = 0.217, P = 0.124), xerosis score (r = 0.206, P = 0.137), oozing/crust score (r = 0.206, P = 0.137), or lichenification score (r = 0.206, P = 0.137) (Table 5).

Similarly, PU.1 expression significantly correlated only with the erythema score (r = 0.360, P = 0.025) and the edema/papule score (r = 0.396, P = 0.015). PU.1 expression did not correlate with any of the excoriation score (r = -0.098, P = 0.304), xerosis score (r = 0.044, P = 0.409), oozing/crust score, (r = 0.044, P = 0.409), or lichenification (r = 0.044, P = 0.409) (Table 5).
IL-9 levels, which related to symptom severity. A single study was reported that atopic dermatitis children have higher serum recognition of other T-cell subtypes as Th17 and Treg. Th2 cells have been well recognized followed later by the AD.23 Th cells are central to AD pathogenesis. The roles of both innate and adaptive immunity have been described in IgE

Hamza et al. PU.1 and IL-9 expression in atopic dermatitis = P < 0.05. These findings have put TH9 into central focus as a possible player in AD pathogenesis. Contradictory results were reported in another study.28 This study, however, focused on systemic sclerosis patients and enrolled AD patients as controls. No details about the age of the patients, severity, or any treatment used was given in the study, making comparison of the study results not possible. Suárez-Fariñas et al. previously reported a significant increase of Th9 related product (IL-9) in lesional intrinsic AD skin of severe AD patients.29

A likely culprit for this elevated IL-9 is Th9 cells. PU.1 expression level in the current study was found to be highest among the AD group. It can be hypothesized that the Th2-cytokine-rich microenvironment of AD triggers a reprogramming process from Th2 cells to Th9 cells contributing to the amplified Th9 cell and IL-9 transcription. Further studies are, however, needed to explore this hypothesis and detect any causal relationship between increased IL-9 and AD and/or disease severity.

Gene expressions of PU.1 and IL-9 in the current study correlated positively with disease severity as assessed by SCORAD index. Ciprandi et al. reported a positive correlation of serum IL-9 with disease severity in children,12 and Ma et al. reported a similar correlation of disease severity to expression levels of PU.1 and IL-9 in AD.13

We showed that PU.1 and IL-9 expression levels were positively significantly correlated. This is in agreement with Chang et al. In their report, PU.1 was identified as a regulator of IL-9 production in T cells. PU.1-deficient T cells had diminished IL-9 production, and ectopic expression of PU.1 increased IL-9 production from both Th2 and TH9 cultures. Also, diminishing PU.1 expression in human IL-9-secreting T-cell cultures was reported to decrease IL-9 production.30 Goswami and Kaplan stated that PU.1 promotes expression of IL-9 by modifying specific histones at the IL-9 locus.31 Results from previous studies have demonstrated that when naive CD4+ T cells were exposed to TGF-β, there was a concomitant increase in PU.1 expression and IL-9 production. Also, ectopic expression of PU.1 converted Th2 cells to Th9 phenotype and enhanced IL-9 production from Th9 cells.32 It is possible that expansion of the Th9 cell subset secondary to upregulation of the PU.1

| Table 5 Correlation between interleukin-9 (IL-9) and PU.1 expression levels and the eruption type and itch score in patients with atopic dermatitis |
|-----------------|-----------------|----------------|----------------|----------------|----------------|
|                 | Erythema        | Edema          | Excoriation     | Lichenification | Xerosis        |
| **IL9**         |                 |                |                |                |                |
| r<sub>s</sub>    | 0.337           | 0.311          | 0.217          | 0.206          | 0.206          |
| P value         | 0.034*          | 0.047*         | 0.124          | 0.137          | 0.137          |
| **PU.1**        |                 |                |                |                |                |
| r<sub>s</sub>    | 0.360           | 0.396          | -0.098         | 0.044          | 0.044          |
| P value         | 0.025*          | 0.015*         | 0.304          | 0.409          | 0.409          |

r<sub>s</sub>, Spearman’s rank-order coefficient.

*Statistically significant at P < 0.05.
transcription factor may lead to increased secretion of the IL-9 cytokine and may contribute to the pathogenesis of AD.

We also observed a positive statistical significant correlation between each of IL-9 and PU.1 gene expression level with serum IgE. These results are in accordance with findings reported by Ma et al.13 These observations support the pathogenic role of IL-9 and PU.1 in AD and suggest a possible function of Th9 cells in AD in another way.

Sehra et al. demonstrated that Th9 cells are a major source of IL-9 in different models of allergic inflammation.33 Th9 cells and Th9 products were also previously found to be highly pathogenic in allergic lung inflammation as well as in some autoimmune conditions.34 In the current study, PU.1 and IL-9 gene expression levels showed no statistical significant difference in AD patients with or without histories of other atopic diatheses. Such finding suggests that the increased gene expression levels are secondary to AD, supporting the existence of a specific pathogenic role of Th9 cells in AD.

To the best of our knowledge, this is the first report of the correlation of the expression levels of PU.1 and IL-9 level with acute phase parameters such as the erythema, edema/papule scores, and lack of correlation with the xerosis, lichenification, oozing, or itch score. In conclusion, based on increased expression level of IL-9 and PU.1 in AD patients compared to controls, it can be concluded that elevated PU.1 and thereby increased Th9 cell transcription and Th9-derived IL-9 could be related to the disease pathophysiology and severity. Yet, further studies are needed to explore any causal relationship between increased IL-9 and AD and/or severity. The most obvious limitation of this study is that PU.1 and IL-9 expression levels were not evaluated after AD treatment. Furthermore, studies conducted with larger numbers of patients are recommended.

References


