

Short communication

Blood eosinophils and serum IgE as predictors for prognosis of interferon-gamma therapy in atopic dermatitis

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Background Interferon-gamma (IFN- γ) therapy has been reported to be effective in atopic dermatitis. However, IFN- γ therapy in atopic dermatitis has not yet been well established. In this study, immunologic variables were evaluated as predictors for the prognosis of IFN- γ therapy in atopic dermatitis.

Methods Sixty-eight atopic dermatitis patients were each treated 18 times with 2×10^6 units/m² IFN- γ . Blood IgE level, eosinophil percentage, eosinophil count, and levels of IFN- γ , interleukin-4 (IL-4), IL-5, and IL-10 were investigated. According to clinical responses, patients were classified into three groups: patients with improved clinical severity scores of over 20% were included in group A; those with improved scores of 20% or less in group B; and those with no improvement in group C.

Results Serum IgE levels and blood eosinophil percentages were the lowest in group A. Most atopic dermatitis patients with an eosinophil percentage over 9% and IgE level over 1500 IU/ml did not respond to IFN- γ therapy. Initial IL-10 levels were the highest in group A. IL-4 levels in group A, and IL-5 and IL-10 levels in all groups were significantly decreased by IFN- γ therapy.

Conclusions IFN- γ therapy may be recommended for atopic dermatitis patients with blood eosinophil percentages less than 9% and serum IgE levels less than 1500 IU/ml.

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Interferon-gamma (IFN- γ) therapy has been reported to be effective in atopic dermatitis by many investigators (1-3). The immunologic abnormalities of atopic dermatitis are characterized by Th1/Th2 imbalance (4), which results in increased interleukin-4 (IL-4) and reduced IFN- γ production (5). IL-4 induces class switching to IgE production in B cells and IFN- γ inhibits this IL-4 effect (6). Increased IL-4 and decreased IFN- γ production in atopic dermatitis promotes IgE synthesis (7). Peripheral blood mononuclear cells (PBMC) from patients with atopic dermatitis have a reduced capacity to produce IFN- γ compared to PBMC from healthy, nonatopic controls (8). Therefore, IFN- γ has been used successfully in the treatment of atopic dermatitis patients. Never-

theless, well-described clinical data have been insufficient, with the result that IFN- γ therapy has not been generally applied. In this study, atopic dermatitis patients were treated with IFN- γ , and they were classified into three groups according to their responses. These groups were analyzed in light of immunologic variables including blood eosinophil percentage and serum IgE levels.

Many studies on cytokines in atopic dermatitis have focused on the capacity of cytokine production. To date, blood Th2 cytokine concentrations have been too low to be regarded significantly in practice. Recently, it has become possible to measure the blood concentrations accurately by using highly sensitive cytokine detection kits. However, there have been no studies as to whether

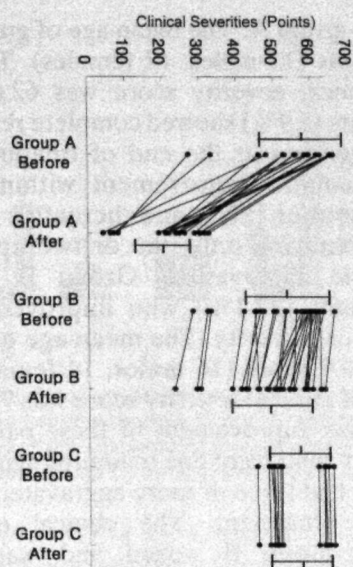


Fig. 1. Grouping of atopic dermatitis according to clinical improvement after IFN- γ therapy in atopic dermatitis. Group A) improvement of over 20% in clinical severity scores; group B) improvement scores of 20% or less; group C) no improvement; before) before IFN- γ therapy; after) after IFN- γ therapy.

blood cytokine levels are clinically significant. Moreover, it is not clear whether blood IFN- γ levels decrease and blood IL-4 levels increase in atopic dermatitis. Cytokines such as IFN- γ , IL-4, IL-5, and IL-10 were studied, and the clinical significance of blood cytokine levels was evaluated in atopic dermatitis.

Material and methods

Patients and controls

Sixty-eight patients with chronic atopic dermatitis were studied. They had severe atopic dermatitis for at least 12 months with no adequate response to topical corticosteroid and systemic antihistamines. The protocol of the study was approved by the ethical committee of Samsung Medical Center in Korea. All patients and normal controls gave written informed consent for all procedures. Diagnostic criteria for atopic dermatitis were those of Hanifin & Rajka (9).

The clinical severity and extent of skin involvement were evaluated on day 0 and day 42 by the scoring system described previously (10). Clinical status was evaluated in light of both objective and subjective criteria. The five parameters of skin involvement were as follows: 1) erythema, edema; 2) vesicles, pustules, crusts; 3) excoriation, cracking; 4) scaling, dryness; and 5) lichenification. These parameters were graded as follows: 0 (none), 1 (mild), 2 (moderate), and 3 (severe). The extent of

the dermatologic manifestations was evaluated in 20 topographic sites: scalp, ears, peribuccal region, periorcular region, the other parts of the face, neck, chest, abdomen, back, elbows (flexures), arms (forearms, axillae, hands), wrists (dorsal), palms (wrists), buttocks (groin), popliteal space, thighs, legs, arches, and soles. A score of 0–3, depending on the percentage of skin surface affected, was assigned for each of these areas. Subjective criteria – pruritus and loss of sleep – were assessed on the scale 0, 100, 200, and 300, and the score was added to a sum of points calculated for all the sites involved as described above.

Patients received subcutaneous injections of 2×10^6 IU/m² recombinant human IFN- γ with a specific activity of 2×10^7 IU/mg (LG Chem, Seoul, Korea). Injections were given for 5 days during the first week of treatment, followed by three injections per week for another 3 weeks, and then followed by two injections per week for the last 2 weeks. Any medications, including corticosteroid and systemic antihistamine, were withheld for at least 4 weeks before the treatment. During the study, local therapy with steroid-free hydrophilic or emollient ointment was allowed. Patients were evaluated before and at the end of treatment with blood sampling. A detailed clinical examination was undertaken by the same investigator, including a quantitative measurement of clinical severity using the scoring system described previously. The following investigations were performed: blood eosinophil percentage, total eosinophil count, and serum IgE. According to the improvement of clinical severity, patients were classified into three groups. Group A comprised patients whose clinical severity scores improved more than 20%, group B comprised patients who showed improvement of 20% or less, and group C comprised patients who showed no improvement. To compare normal blood cytokine levels, we studied 30 normal subjects who visited our hospital for a periodic health examination, had no family history or past medical history of allergic diseases, and had no illness at the time of test.

Plasma IFN- γ , IL-4, IL-5, and IL-10 assay

For cytokine assay, 2 ml of heparinized peripheral venous blood was drawn, and a sample was layered onto an aliquot (5 ml) of Ficoll-Paque (Pharmacia, Uppsala, Sweden) in a sterile conical tube (Falcon, Becton-Dickinson, Cowley, UK). After centrifugation at 1000 g for 20 min at 20°C, plasma was collected for analysis of cytokines. The plasma samples were stored at –80°C until they were tested for plasma cytokine concentrations, which were measured with the highly sensitive IL-4 ELISA kit

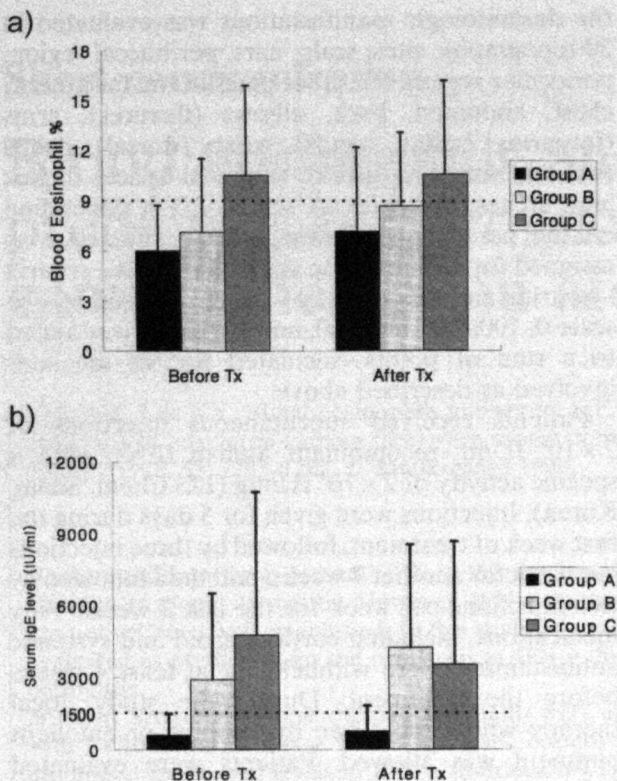


Fig. 2. a) Initial blood eosinophil percentage in subgroups of atopic dermatitis; b) initial serum IgE levels in subgroups of atopic dermatitis. Group A) improvement of over 20% in clinical severity scores; group B) improvement scores of 20% or less; group C) no improvement; before Tx) before IFN- γ therapy; after Tx) after IFN- γ therapy.

(sensitivity, 0.09 pg/ml; range, 0.25–16 pg/ml) (R&D, Minneapolis, MN, USA), the IL-5 ELISA kit (sensitivity, 3 pg/ml; range, 7.8–500 pg/ml) (R&D), the high sensitivity IL-10 ELISA kit (sensitivity, 0.5 pg/ml; range, 0.781–50 pg/ml) (R&D), and the IFN- γ ELISA kit (sensitivity, 2 pg/ml; range, 9.4–600 pg/ml) (R&D). These procedures were carried out according to the manufacturer's directions.

For analysis of the significance of the difference among groups, statistical comparisons were made throughout this study with the Wilcoxon matched-pair signed-rank test and the Mann-Whitney U-test. The results were expressed as mean $\pm$ SD.

Results

Clinical response

Clinically, 53 atopic dermatitis patients (77.9%) were improved by IFN- γ therapy (Fig. 1). Patients were classified into three groups, and each group showed its own characteristic patterns of clinical responses (Fig. 2). Twenty-three patients (33.8%) improved over 20% in clinical severity and were

included in group A. The mean age of group A was 9.7 ± 9.2 years (11 males, 12 females). The reduction of clinical severity score was $62.6 \pm 12.4\%$. Four patients (5.9%) showed complete remission of clinical symptoms at the end of treatment. They showed dramatic improvement within the first four injections of IFN- γ and thereafter improved gradually, showing only one or two episodes of symptomatic aggravation. Group B consisted of 30 patients (44.1%) who improved 20% or less in clinical severity. The mean age of group B was 13.0 ± 9.9 years (16 males, 14 females). The reduction of clinical severity score was $7.8 \pm 5.7\%$. However, the improvement of these patients was not clinically significant due to limited symptomatic relief. They had three or more aggravated episodes during the treatment. The clinical course of patients in group B waxed and waned. The remaining 15 patients (22.1%) showed no improvement and were included in group C. The mean age of group C was 9.7 ± 9.2 years (eight males, seven females). There were no significant differences in the initial clinical severities among group A (566.1 ± 104.8 points), group B (538.6 ± 98.3 points), and group C (560.3 ± 76.8 points) ($P > 0.05$).

Laboratory findings

Eosinophil percentage and count. After IFN- γ therapy, mean eosinophil percentage increased insignificantly from $6.0 \pm 2.7\%$ to $7.2 \pm 5.0\%$ ($P > 0.05$) in group A, from $7.1 \pm 4.4\%$ to $8.7 \pm 4.4\%$ ($P > 0.05$) in group B, and from $10.5 \pm 5.4\%$ to $10.6 \pm 5.6\%$ ($P > 0.05$) in group C. Initial eosinophil percentage was significantly lower in group A than in group B ($P < 0.05$), and in group B than in group C ($P < 0.05$) (Fig. 2). Eosinophil count decreased insignificantly from $559.1 \pm 268.3/\mu\text{l}$ to $555.0 \pm 343.8/\mu\text{l}$ ($P > 0.05$) in group A, from $732.6 \pm 407.4/\mu\text{l}$ to $555.0 \pm 343.8/\mu\text{l}$ ($P > 0.05$) in group B, and from $866.7 \pm 791.1/\mu\text{l}$ to $555.0 \pm 343.8/\mu\text{l}$ ($P > 0.05$) in group C. Initial eosinophil count was significantly lower in group A than group C.

Serum IgE levels. After IFN- γ therapy, serum IgE level significantly increased from 593.1 ± 891.9 IU/ml to 756.7 ± 1058.3 IU/ml ($P < 0.05$) in group A, and from 2912.5 ± 3584.2 IU/ml to 4240.3 ± 4292.3 IU/ml ($P < 0.05$) in group C (Fig. 2b). However, serum IgE levels of group B decreased insignificantly from 4768.9 ± 5952.3 IU/ml to 3554.2 ± 5121.0 IU/ml ($P > 0.05$). Initial serum IgE level was significantly lower in group A than those in groups B and C ($P < 0.001$).

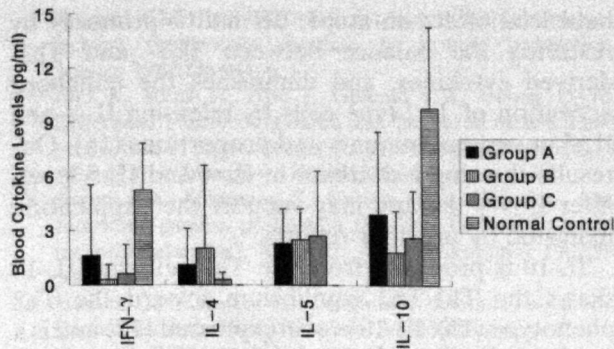


Fig. 3. Initial blood cytokine levels of subgroups of atopic dermatitis and normal controls. Group A) improvement of over 20% in clinical severity scores; Group B) improvement scores of 20% or less; group C) no improvement.

Plasma IFN- γ , IL-4, IL-5, and IL-10 levels. Initial plasma IFN- γ levels were 1.6 ± 3.9 pg/ml in group A, 0.3 ± 0.8 pg/ml in group B, and 0.6 ± 1.6 pg/ml in group C. These were lower than that of normal controls (5.2 ± 2.6 pg/ml) ($P < 0.05$) (Fig. 3). Initial IFN- γ levels of these three groups showed no difference statistically ($P > 0.05$). Initial IL-4 levels were 1.1 ± 2.0 pg/ml in group A, 2.0 ± 4.6 pg/ml in group B, and 0.3 ± 0.4 pg/ml in group C. Initial IL-5 levels were 2.3 ± 1.9 pg/ml in group A, 2.5 ± 1.7 pg/ml in group B, and 2.7 ± 3.6 pg/ml in group C. IL-4 and IL-5 increased in atopic dermatitis, while they were not detected in normal controls. The initial IL-4 level in group C was significantly lower than those in groups A and B. Initial IL-5 levels in these three groups showed no difference statistically. Initial IL-10 levels were 3.9 ± 4.6 pg/ml in group A, 1.8 ± 2.3 pg/ml in group B, and 2.1 ± 2.6 pg/ml in group C. Interestingly, initial IL-10 levels of atopic dermatitis patients were rather lower than those of normal controls (9.8 ± 4.5 pg/ml) ($P < 0.05$). Initial plasma IL-10 level was the highest in group A. IFN- γ levels remained unchanged statistically in all groups after IFN- γ therapy (group A, 1.2 ± 3.9 ; group B, 0.1 ± 0.1 ; group C, 2.0 ± 6.0). IL-4 level significantly decreased only in group A ($P < 0.05$) (group A, 0.5 ± 0.6 ; group B, 1.0 ± 1.5 ; group C, 0.2 ± 0.2). IL-5 (group A, 0.3 ± 0.8 ; group B, 0 ± 0 ; group C, 0 ± 0) and IL-10 levels (group A, 2.1 ± 5.6 ; group B, 0.3 ± 0.8 ; group C, 0.5 ± 1.1) were significantly lowered in all groups ($P > 0.05$).

Discussion

Three types of IFN- γ responses in atopic dermatitis are presented in this study: groups A and B (response group) and group C (nonresponse group). The 77.9% of patients responding to IFN- γ therapy were separated into group A and group B according to the clinical course, frequency of

aggravation episodes, and reduction of clinical severity scores (Fig. 1). Each group showed three characteristic patterns of clinical responses. Group A showed rapid improvement within the first four injections of IFN- γ and had only one or two episodes of aggravation. However, group B showed limited improvement, frequent episodes of aggravation, and insignificant clinical relief, resulting in improved scores of 20% or less. In groups A and B, the exact causes of aggravation were not known in many patients. In some patients, aggravation episodes were accompanied by the ingestion of a certain food, sweating, menstruation, exposure to heavy dust, and upper respiratory infection. This clinical information suggests that atopic dermatitis should not be considered a homogeneous disease. Therefore, it is unreasonable to expect all atopic dermatitis patients to respond to a single drug, including IFN- γ . It is also important to control related factors such as food and dust simultaneously with IFN- γ therapy.

Clinically, the results of IFN- γ therapy could be predicted simply by the patterns of clinical course and the frequency of aggravation episodes. The present investigation was performed as an uncontrolled study without placebo control, and this was a limitation of our study. However, the purpose of this study was to search for the simple indicators of good responders to IFN- γ therapy with immunologic parameters, as the effectiveness of IFN- γ in atopic dermatitis is well known. As the analysis was conducted among the patients with the same clinical conditions, all patients in group A may be regarded as the patients showing favorable responses. This study does not rule out the possibility that patients in groups B and C may be further improved if treated with higher-dose IFN- γ or for longer periods. Further study about the effects of IFN- γ dosage changes or length of treatment periods will be needed. No particular side-effects, except mild fever, of the treatment were discovered in the present study. Mild febrile reactions of the three groups showed no significant difference.

Blood eosinophil percentage and serum IgE level were analyzed retrospectively as predictors of the prognosis of IFN- γ therapy for atopic dermatitis in the three groups (Fig. 2a). Blood eosinophil counts showed no significant difference among the three groups and remained unchanged despite IFN- γ therapy. The maximum blood eosinophil percentage was lower than 9% in group A, and mean blood eosinophil percentages were over 10% in group C. Eosinophilia is known to be correlated with the clinical severity of atopic dermatitis (11). Blood eosinophil percentage may be regarded as an important factor in determining

IFN- γ responsiveness. In addition, serum IgE level is another important indicator for allergic diseases. Serum IgE level is elevated in atopic dermatitis, as in other allergic diseases. Maximal serum IgE level in group A was less than 1500 IU/ml initially, and mean serum IgE levels were over 2000 IU/ml in groups B and C (Fig. 2b). Initial serum IgE level may also be an important factor in predicting the results of IFN- γ therapy in atopic dermatitis. Therefore, IFN- γ therapy may be recommended in patients with an eosinophil percentage of less than 9% and with serum IgE levels of less than 1500 IU/ml.

Most studies on IFN- γ and IL-4 in atopic dermatitis have focused on the capacity of cytokine production. The clinical significance of blood cytokine levels has not been evaluated exactly due to the extremely low blood concentrations. Recently, highly sensitive cytokine ELISA kits have become available, of which the sensitivity was improved by up to 0.09 pg/ml. In this study, IFN- γ levels actually decreased in atopic dermatitis compared to those of normal subjects (Fig. 3). While IL-4 and IL-5 were not detected in normal subjects, IL-4 and IL-5 were quantitatively detectable in atopic dermatitis. In previous reports, IL-5 levels were above 40 pg/ml in atopic patients with glucocorticoid-dependent chronic asthma (12). In this study, IL-5 levels measured less than 15 pg/ml. Despite the IL-5 levels in both results, it is noteworthy that IL-5 was quantitatively detected in both atopic dermatitis and chronic asthma. Interestingly, the initial plasma IL-4 level in group C was significantly low compared to those in groups B and C. This result was inconsistent with the theoretic background. Therefore, group C may be regarded as a different subtype of atopic dermatitis, as described above. The IL-4 level of group A was decreased by IFN- γ therapy. Plasma IL-5 and IL-10 levels were decreased significantly by IFN- γ therapy. The reduction of IL-4, IL-5, and IL-10 levels may represent inhibitory effects of IFN- γ on Th2 blood cytokine levels.

Although it was originally hypothesized that IFN- γ would be beneficial for atopic dermatitis because of its ability to downregulate Th2 responses, there was no reduction in spontaneous IgE production (13). The clinical improvement of atopic dermatitis was not necessarily associated with a decrease in serum IgE levels (14). Serum IgE level was reported to be reduced by IFN- γ therapy by other investigators (2, 3). In our study, IgE levels were decreased by IFN- γ therapy and rather increased in groups A and C. In spite of the increase in serum IgE levels, atopic dermatitis patients were improved by IFN- γ therapy. It is tempting to speculate that IFN- γ exerts its

beneficial effect on atopic dermatitis primarily by restoring the balance between Th1- and Th2-derived cytokines, and diminishes the enhanced activation of Th2-type cells by releasing IL-4 and IL-5 in various amounts and proportions (15). Our results showing a decrease in IL-4 and IL-5 levels after IFN- γ therapy may support the explanation indicated by previous reports.

IL-10 is produced from the Th2 cell, and IL-10 skews the Th1/Th2 equilibrium toward the Th2 phenotype (16). IL-10 is overexpressed (17) and is a predominant cytokine in atopic dermatitis (18). Therefore, the IL-10 level was expected to be high, but was found to be rather low in atopic dermatitis compared to normal subjects in this study. Moreover, initial levels were significantly high in group A compared to those in groups B and C. High IL-10 levels seemed to be a favorable factor in IFN- γ therapy in atopic dermatitis. IL-10 is an essential factor for B-cell differentiation (19) and is known to inhibit IgE production in atopic dermatitis at the same time (20). In this way, a reduction of IL-10 by IFN- γ therapy may lead to the elevation of serum IgE levels. There is a report that IFN- γ has downregulating effects on IL-4 and IL-10 (21). These effects may be reflected in the decrease of IL-10 levels in this study.

In conclusion, IFN- γ therapy showed three characteristic patterns of clinical response. IFN- γ therapy may be recommended for atopic dermatitis patients with a blood eosinophil percentage of less than 9% and a serum IgE level under 1500 IU/ml. Clinically, these patients were expected to show a clinical course of rapid improvement with fewer than four injections of IFN- γ and fewer than three aggravated episodes. These patients were expected to be improved significantly by IFN- γ therapy, and further investigations of the clinical application of IFN- γ therapy in atopic dermatitis may be needed.

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