

Short communication

Interferon-gamma in the treatment of atopic dermatitis: influence on T-cell activation

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Atopic dermatitis (AD) is a chronic relapsing skin disease characterized by various immunologic abnormalities. Its pathogenesis remains obscure and its treatment difficult. We have studied the efficacy of systemic recombinant human interferon-gamma (rhIFN- γ) treatment (0.95 mg m⁻² sc on 3 consecutive days, during 4 weeks) in 10 patients with severe AD. Marked clinical improvement was observed starting from the third week of treatment. Erythema, dryness, and lichenification were the most responsive symptoms. Serum immunoglobulin E and IgG4 levels did not change during treatment. Blood eosinophil count decreased only transiently at the end of the first and second series of injections (days 4 and 11; $P = 0.02$). Patients with AD showed an increase in CD25-positive cells (11.0% vs 4.88%; $P = 0.0001$) as compared to 10 age-matched healthy controls. Moreover, in parallel with clinical improvement, a distinct decrease in CD25-positive lymphocytes was observed on days 32 and 50 ($P = 0.002$ and $P = 0.006$, respectively). We suggest that in AD the beneficial effect of rhIFN- γ might be related to the inhibition of excessive T-cell activation, perhaps of the subpopulations, producing interleukin (IL)-4 and IL-5.

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Atopic dermatitis (AD) is a troublesome inflammatory skin disease characterized by severe pruritus, typical eczematous morphology, and a chronic relapsing course (7). The pathogenesis of AD remains uncertain. The disease is accompanied in 60–80% of patients by elevated total serum immunoglobulin E (IgE) levels (7). It has been suggested that increased lymphocyte interleukin (IL)-4 and/or impaired IFN- γ production are at least partially responsible for the increased IgE synthesis (10, 22).

For these reasons, recombinant human interferon-gamma (rhIFN- γ) has been used in the treatment of AD patients. However, the clinical improvement observed in these patients was not necessarily associated with a decrease in total serum IgE levels (9, 16). Recent evidence indicates that chronic skin inflammation is likely to result from helper T-cell (probably atopy-specific Th2-like cell) and eosinophil involvement (12). Skin lesions in AD show infiltration by various types of activated T cells, and antigen-

presenting Langerhans' cells (1), together with deposits of eosinophil major basic protein (MBP) and eosinophil cationic protein (ECP) (3). In the peripheral blood of these patients, there were observed increased numbers of eosinophils, changes in activated CD4 + T-cell and CD8 + T-cell numbers, and elevated serum levels of eosinophil cationic protein (7, 14, 18).

Therefore, we decided to study the clinical effects of rhIFN- γ given for 4 weeks in 10 patients with the severe form of AD, focusing our attention on serum IgE and ECP levels, whole blood eosinophil counts, and major classes of lymphocyte immunophenotypes and their lymphocyte surface-activation markers.

Material and methods

Ten patients with AD (eight women and two men), aged 18–36 (mean 25) years, were enrolled in the open study. All participants fulfilled the standard

criteria of Hanifin et al. for AD (8). Systemic and local steroids as well as antihistamines were withheld for at least 3 weeks before the beginning of the study. Local therapy with steroid-free hydrophilic or emollient ointments was allowed.

rhIFN- γ (Imukin, Boehringer Ingelheim, Germany) was administered subcutaneously in the single daily dose of 0.05 mg m⁻², for 3 consecutive days for 4 weeks on an outpatient basis. Patients were permitted to take 1000 mg of acetaminophen orally in case of adverse reactions.

The clinical severity and extension of skin involvement were evaluated on days 0, 11, 17, 25, 32, and 50 by the scoring system developed by Costa et al. (4). The final score was defined as the sum of all points for objective and subjective criteria. Five parameters of skin involvement: 1) erythema, edema; 2) vesicles, pustules, crusts; 3) excoriation, cracking; 4) scaling, dryness; 5) lichenification) 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, face (other), neck, chest, abdomen, back, elbows (flexures), arms – forearms, axillae, hands – wrists (dorsal), palms – wrists, buttocks – groins, 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.

Blood for laboratory determinations was taken on days 0, 4, 11, 25, 32, and 50, unless otherwise stated. Serum total IgE was measured by the nephelometric method (Behringwerke, Marburg, Germany). Serum IgG4 concentration (radial immunodiffusion; Binding Site, UK) and serum eosinophil cationic protein (ECP) levels (radioimmunoassay; Pharmacia, Sweden) were measured on days 0, 4, 25, and 50.

Lymphocyte subpopulations were analyzed by flow cytometry (FACStar Plus, Becton Dickinson,

USA) using monoclonal FITC conjugated antibodies against CD4, CD8, CD19, CD25, and HLA-DR (Dakopatts AB, Denmark) (5). Ten non-atopic healthy age-matched volunteers served as controls.

Data were analyzed using the Wilcoxon sign-rank test, and results were expressed as means $\pm$ SD.

Results

A significant clinical improvement was observed in all patients. Severity criteria scores (with the exception of the presence of vesicles, pustules, and crusts) were significantly reduced starting from day 17. The greatest and longest-lasting beneficial effect of IFN- γ administration (up to day 50) was observed for scaling, dryness, and lichenification of the affected skin (Table 1). The improvement occurred, especially in some topographic sites – peribuccal and periorcular regions, neck, abdomen, back, and elbows ($P < 0.05$). Oddly enough, pruritus revealed no trend to subside during the study. No major adverse reactions were observed during treatment, and all subjects completed the trial.

As could be expected, serum total IgE levels were elevated in the AD patients studied (geometric mean: 796.3 IU/ml). However, those levels remained unchanged during IFN- γ treatment. There was no change in the level of IgG4 (Table 1). The moderately elevated whole blood eosinophil count significantly decreased on days 4 and 11 of IFN- γ therapy (from 424.4 $\pm$ 392.3 cells mm⁻³ to 179.2 $\pm$ 149.4 cells mm⁻³ ($P = 0.019$) and 241.9 $\pm$ 289.6 cells mm⁻³ ($P = 0.025$), respectively). Subsequently, eosinophilia began to rise, reaching initial values at the end of the study (Table 1). Serum ECP levels showed an insignificant decrease also on days 4 and 25 (Table 1).

The baseline CD4, CD8, CD19, and HLA-DR lymphocyte cell subpopulations and the CD4/CD8 ratio revealed no differences between patients and controls. However, CD25-positive T cells were markedly increased in patients as compared to normal individuals (average 11.03% vs 4.88%, $P =$

Table 1. Influence of IFN- γ on total score, blood eosinophil count, IgG4, ECP, IgE and CD25+ lymphocytes in AD patients (mean $\pm$ SD)

Days	Total score	Blood eosinophil count (cells mm ⁻³)	IgG4* (µg/l)	ECP ^b (µg/l)	IgE (IU/l) (geometric mean $\pm$ SE)	CD25 (%)
0	653.6 $\pm$ 545.2	424.4 $\pm$ 392.34	496.4 $\pm$ 115.0	16.5 $\pm$ 7.9	795.3 $\pm$ 1161.1	11.03 $\pm$ 2.7
4	511.3 $\pm$ 329.9	179.2 $\pm$ 149.44*	473.3 $\pm$ 120.9	11.7 $\pm$ 4.7	783.9 $\pm$ 1067.2	13.30 $\pm$ 3.25
11	356.9 $\pm$ 200.1*	241.9 $\pm$ 239.55*	493.1 $\pm$ 109.9	Not measured	976.1 $\pm$ 1277.4	12.93 $\pm$ 3.45
25	356.3 $\pm$ 251.2*	353.3 $\pm$ 411.59	503.4 $\pm$ 150.7	10.7 $\pm$ 5.9	895.7 $\pm$ 1210.3	10.21 $\pm$ 4.43
32	309.0 $\pm$ 172.3*	312.7 $\pm$ 262.35	504.6 $\pm$ 156.8	Not measured	795.2 $\pm$ 1177.8	5.33 $\pm$ 1.27*
50	421.6 $\pm$ 333.3	484.1 $\pm$ 721.97	Not measured	17.4 $\pm$ 13.4	855.3 $\pm$ 908.4	5.5 $\pm$ 3.03*

* $P < 0.05$ as compared to day 0.

^a Recommended normal range: 0.1–0.9 mg/l.

^b Recommended normal values: below 16 µg/l.

0.00001). Treatment with IFN- γ had no influence on CD4, CD8 lymphocyte subsets and the CD4/CD8 ratio. In contrast, CD19- and HLA-DR-positive T-cell populations decreased significantly on day 32 of the study (from 6.7%, to 4.5%, and from 15.7%, to 11.5%, respectively; $P < 0.036$). Moreover, the number of cells expressing CD25 diminished markedly at the final phase of the trial, i.e., on observation days 32 and 50 ($P = 0.002$ and $P = 0.006$, respectively) (Table 1).

Discussion

In this open clinical trial, we have shown that patients with AD could benefit from treatment with rhIFN- γ given subcutaneously for 4 weeks. This finding is in agreement with the results of other reports (2, 9, 15, 16).

We failed to detect any significant changes in serum IgE levels during IFN- γ treatment. This confirms the results of previous studies (2, 9, 15) and supports the view that elevated concentrations of IgE might be of minor importance in the pathogenesis of AD (9).

Typically elevated peripheral blood eosinophil count decreased only transiently during the first 2 weeks of therapy with rhIFN- γ . This change was not accompanied by a significant decrease in serum ECP levels. We are aware of only one other study in which the peripheral blood eosinophil count has been assessed in AD patients during IFN- γ treatment (9). In this study, eosinophil numbers were significantly decreased up to week 12 of treatment. This difference between the two studies might be explained by a lower weekly dose of the drug used in our trial. However, we have observed clinical improvement despite the return of eosinophil count to baseline values and unchanged serum ECP levels.

Lymphocyte immunophenotypes did not reveal any significant changes in the subsets of CD4+ and CD8+ cells or their ratio. Results of previous studies were conflicting. Several reports demonstrated reduced numbers of CD8+ cells and sometimes increased numbers of CD4+ cells (13, 14, 18, 21). However, the intriguing finding of our study was an increased number of circulating activated (IL-2-receptor-bearing, CD25-positive) lymphocytes. After IFN therapy, the number of activated lymphocytes (CD25-positive) decreased promptly and in parallel with clinical improvement. Similar peripheral blood lymphocyte activation in AD patients has been recently observed by Walker et al. (21). They and others (17) have also shown a decrease of activated lymphocytes numbers when a clinical improvement has been achieved using various therapeutic approaches. These activated lymphocytes probably belong to the Th2-like cells and are a major pathogenic

factor in AD (11, 20). In these patients, reduced IFN- γ secretion by isolated peripheral blood mononuclear cells could be observed (19, 22).

Thus, it is tempting to speculate that IFN- γ exerts its beneficial effect in AD primarily by restoring the balance between Th1 and Th2-derived cytokines and, in this way, diminishes the enhanced activation of Th-2-type cells, releasing IL-4 and IL-5 in various amounts and proportions (6).

It is most likely that IFN- γ also influenced B-lymphocyte population, as shown by the parallel decrease of CD19- and HLA-DR-positive cells during treatment. The single-immunofluorescence technique used did not support this finding.

In conclusion, rhIFN- γ is an efficacious novel therapeutic approach in patients with severe AD. Moreover, the beneficial effect of IFN- γ might be primarily mediated by reducing the number of lymphocyte subsets showing markers of cell activation.

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