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The Influence of Interferon-gamma and Interleukin-4 on IgE Production in B Lymphocytes of Patients with Atopic Dermatitis

A Possible Criterion for Selection of Patients for Interferon Therapy

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The regulation of IgE production in B lymphocytes of patients with atopic dermatitis by interleukin-4 (IL-4) and interferon-gamma (IFN-gamma) was studied. IL-4 stimulated IgE production in vitro in B-cells of healthy donors and of children with atopic dermatitis, but had only a marginal effect on the high basal level of IgE production by lymphocytes from adult patients with atopic dermatitis. The addition of IFN-gamma prevented in all cases the stimulation of IgE synthesis induced by IL-4. The production of IgG and IgM was differently influenced. These results indicate that the in vitro production of IgE by mononuclear cells from adult patients is more resistant to the regulatory effects of IL-4 and IFN-gamma than is that in B cells of children with atopic dermatitis. We propose that a previous in vitro test of the responsiveness of IgE-producing B cells to IFN-gamma may be used to select patients with atopic dermatitis for treatment with IFN-gamma.

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Even though atopic dermatitis (AD) is a common disease, its etiology is not yet fully explored. Patients with AD often have high concentrations of IgE in serum and its synthesis in vitro by their B cells is increased (1). IgE receptor-bearing epidermal Langerhans cells are involved in the generation of skin lesions (2). Other disturbances have been described, such as increased phosphodiesterase activity and reduced expression of β-adrenergic receptors, leading to reduced concentrations of cyclic adenosine monophosphate, and cholinergic hyperreactivity (3).

In the regulation of IgE synthesis by B lymphocytes, several agents seem to play a role, such as specific T-helper and functional T-suppressor cells and their factors (4), anti-IgE antibodies (3,5), and IgE-binding factors (6). Two subclasses of T-helper cells may exist: (i) Th_2 cells, producing IL-4 and IL-5, that selectively stimulate B-cells, releasing IgE and IgA, respectively; (ii) Th_1 cells, on the other hand, produce among other mediators IFN-gamma, which counteracts the effect of IL-4 on B cells (7,8). IL-4 causes an increase in IgE synthesis and expression of F_ε-receptors (CD23) specific for IgE (9). In the human system, no such T-helper cell subpopulations could readily be found (10), though recent reports have established that, in vivo, primed T cells can produce a particular (e.g. Th_1- or Th_2-like) lymphokine pattern (11) as could be demonstrated at the single T-cell level (12).

On the basis of experimental data, both IFN-gamma (13,14) and IFN-alpha (15,16) were used to treat patients with AD (13,14,15) and with hyper-IgE syndrome (16). In a significant proportion of the cases, the concentration of IgE in serum was reduced and an improvement of clinical symptoms was achieved.

In this work we investigated whether the above described regulator mechanisms operate in lymphocytes of patients with AD. For this purpose we cultured peripheral blood mononuclear cells of healthy donors and of children and adult patients suffering from AD in the presence of IL-4, IFN-gamma, and both mediators together. The synthesis of IgE, IgG and IgM was determined. This study shows that the responsiveness of IgE-producing B cells to IFN-gamma is preserved in most children with AD and in a proportion of adult patients (30%). We propose the use of this in vitro system to select AD patients for therapy with IFN-gamma.

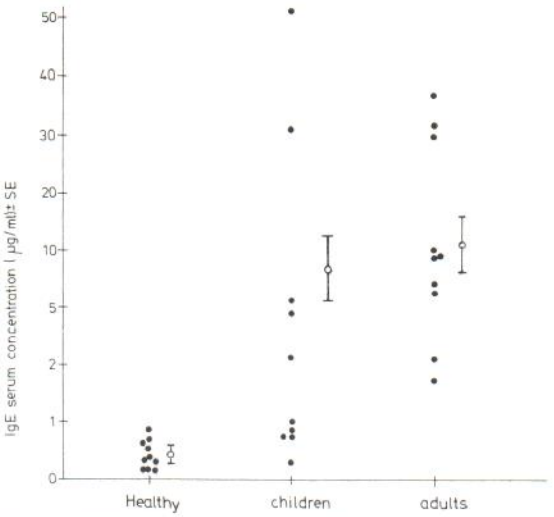

Fig. 1. Serum concentrations of IgE in healthy individuals and in child and adult patients with AD.

PATIENTS AND METHODS

From among patients at the Department of Dermatology, we selected 10 children (age range: 2–12 years) and 10 adult patients (aged 16–59 years), suffering from severe AD. They were not treated at least for 3 months prior to the study with internal corticosteroids. Disease activity was assessed by the atopic eczema activity and severity index (AEASI) which was calculated as described by Hanifin & Rajka (17). As controls we chose healthy individuals, not suffering from AD or other atopic disease (aged 26–51 years).

Peripheral blood obtained by venepuncture was supplemented with 10 µg/ml heparin (Gedeon Richter, Budapest, Hungary). To isolate mononuclear cells the blood was mixed with an equal part of phosphate-buffered saline and centrifuged for 40 min at 400 *g* over a density gradient (1.077 kg/l), consisting of Ficoll® (Pharmacia, Uppsala, Sweden) and Visotrast® (Fahlberg-List, Magdeburg, GDR). The mononuclear cells sedimenting over the density gradient were resuspended in phosphate-buffered saline and washed twice by centrifugation for 15 min at 300 *g*. Finally the cells were suspended in RPMI 1640 medium (Serva, Heidelberg, FRG), which was supplemented with 10% heat-inactivated fetal calf serum, 2 mM glutamine, 15 mM HEPES buffer and 10 µg/ml gentamycin. 0.2 ml aliquots of the suspension, containing 2×10^6 cells per ml, were cultured for 8 days on microplates in a humidified atmosphere containing 5% CO_2 at 37°C in the presence of cytokines, as indicated. The cells were centrifugated and IgM, IgG and IgE concentrations were determined in the supernatants.

Recombinant human IFN-gamma (kindly provided by Dr D. Lando, Roussel-UCLAF, France) having a specific activity of 2×10^7 U/mg, was used at a concentration of 100 U/ml. As a source of IL-4, two different preparations of recombinant human IL-4 were used at a concentration of 100 U/ml (Genzyme, Boston, Mass., USA, and Ciba Geigy Ltd., Basle, Switzerland).

The ELISA assays for IgG/IgM were performed as sandwich assays using affinity purified goat anti-human IgG/IgM antibodies as coating and peroxidase-labelled reactants. The IgE sandwich ELISA was based on two monoclonal anti-human IgE antibodies (TN-142 and 2-1-5), originally selected and provided by Dr G. Delespesse and Dr K. Turner, respectively. The sensitivity of the IgG and IgM ELISA was about 1 ng/ml, and of the IgE assay, 0.1 ng/ml.

For statistics, linear regression and the non-parametric Mann–Whitney test were used.

RESULTS

In the present study, three groups of 10 individuals each were investigated: healthy donors, and both child and adult patients suffering from AD. At the time of the study, the patients showed no clinical symptoms of respiratory allergy. Two children and one adult patient had a history of episodes of asthmatic bronchitis.

Fig. 1 shows elevated serum levels of IgE in most AD patients, whether children or adults. Furthermore, a significant correlation was found between the disease activity (AEASI) and serum IgE concentration ($r = 0.56$, $p < 0.05$). When mononuclear cells were cultured alone or in the presence of IL-4, basal IgE production increased more in adult patients than in children with AD (Fig. 2). In children with AD, no correlation was found between serum IgE concentration and in vitro IgE production ($r = 0.0941$), but in adult patients both parameters showed a positive correlation ($r = 0.6473$, $p < 0.05$). There was no correlation in vitro between IgE synthesis and disease activity. IL-4 stimulated IgE production by cells from healthy donors ($p < 0.01$) and

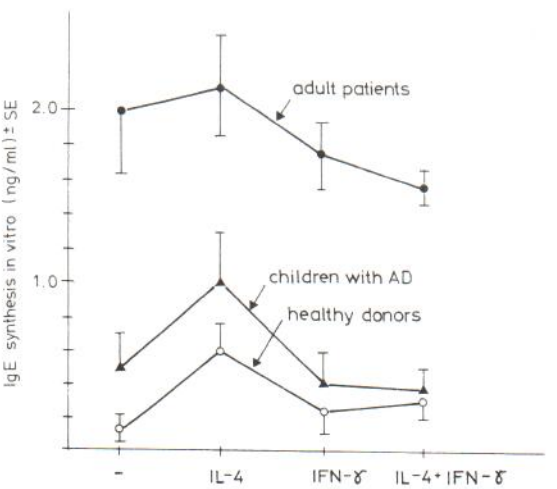

Fig. 2. Regulation of IgE synthesis in vitro by IL-4 and IFN-gamma.

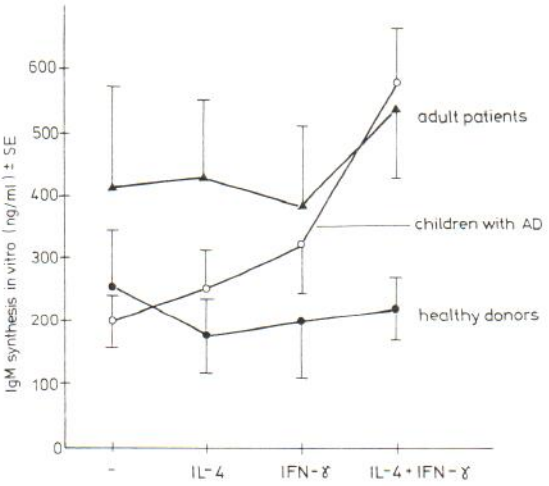

Fig. 3. Influence of IL-4 and IFN-gamma on IgM synthesis in vitro.

children with AD ($p < 0.05$) but had no significant effect on IgE synthesis by lymphocytes from adult AD patients. Increased IgE production was stimulated by IL-4 in 10/10 healthy donors, in 8/10 children with AD and in 3/10 adult AD patients. IFN-gamma alone had no significant effect on IgE synthesis, but was able to completely reverse the stimulation of IgE production, induced by IL-4 in all cases, where it was effective. When IL-4 and IFN-gamma were added simultaneously, no statistically significant difference was observed vis-à-vis the group without lymphokine supplementation.

Fig. 3 depicts the synthesis of IgM in vitro. Basal IgM production was also significantly increased in adult AD patients ($p < 0.05$), whereas IL-4 and IFN-gamma had no effect, though in mononuclear cells of healthy donors, both mediators together increased IgM synthesis ($p < 0.01$). Fig. 4 shows IgG production in the same cultures; basal production was increased in cells of adult AD patients ($p < 0.05$), but the effects of IL-4 and IFN-gamma (alone or together) were not significant.

These results show that the susceptibility of IgE-producing B cells to the regulatory influence of IL-4 and IFN-gamma persists in children with AD, but only to a certain extent in adult AD patients.

DISCUSSION

In this work we compared the regulatory influence of the lymphokines IL-4 and IFN-gamma on IgE production in B lymphocytes of patients with AD and from healthy individuals. For this purpose we cultured mononuclear cells of healthy donors and from child and adult patients suffering from AD, by adding IL-4, IFN-gamma, or both mediators together. The synthesis of IgE, IgM and IgG was determined after 8 days of culture. We found a statistically significant correlation of the disease activity (AEASI) *only* with serum IgE concentration, but not with in vitro synthesis of IgE. This may indicate that IgE-producing B lymphocytes of adult patients are preactivated in vivo by IL-4 or other factor(s); no further stimulation could be achieved with IL-4 in vitro. In children this preactivation may have occurred only partially, and in healthy individuals, not at all. IFN-gamma alone had no significant effect on IgE synthesis, but the fact that it reversed the stimulation of IgE synthesis by IL-4 in all cases where it was effective is a significant finding. This shows that IFN-gamma counteracts the stimulatory action of IL-4 on IgE-producing B cells, but when this stimulation had already occurred it could not be reversed by IFN-gamma.

The results, depicted in Figs. 3 and 4, show that the production of IgM and IgG is differently regulated by IL-4 and IFN-gamma, thus demonstrating the IgE-specific effects of these two lymphokines. Remarkably, lymphocytes from adult patients with AD also showed a spontaneous, copious secretion of IgM and IgG, suggesting a general hyperreactivity of B-cells and/or T-helper cells in this disease. In contrast to their antagonistic effects on IgE synthesis,

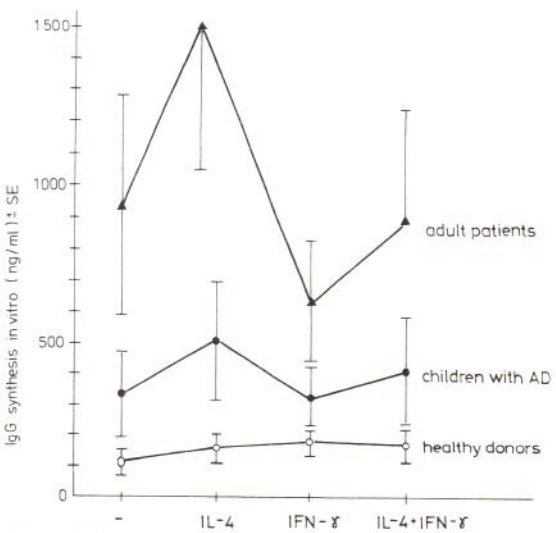

Fig. 4. Influence of IL-4 and IFN-gamma on IgG production in vitro.

IFN-gamma and IL-4 had a synergistic action on IgM secretion by lymphocytes from healthy donors.

Summarizing the results obtained, we can conclude that IgE-producing B lymphocytes from young patients with AD had not lost their susceptibility to the regulatory influence of IL-4 and IFN-gamma. The increased concentrations of IgE in serum and in cultures of mononuclear cells from AD patients may be the result of activation of IgE-specific B cells in vivo. IFN-gamma was able to prevent the stimulation of IgE-producing B cells by IL-4, but could not reverse this activation if it had already occurred, as often observed in adult AD patients. The reason for these different responses of lymphocytes from young vs. adult AD patients is not clear. In lymphocytes of patients with AD a diminished production of IFN-gamma in vitro was found, which was inversely correlated to serum IgE concentration (18,19). This demonstrates that the activation of IgE-producing B lymphocytes in vivo may be the result of a diminished production of IFN-gamma. It is known that IL-4 is able to suppress IFN-gamma production and vice versa (12). In mice, two T-helper subsets secreting alternative lymphokine patterns (IL-4 versus IFN-gamma) have been described (7,8). Different antigen-presenting and co-stimulatory signals seem to determine which T-helper cell subset will be activated. Therefore it was postulated that an imbalance of these two pathways results in atopic disorders, for instance AD. The repeated occurrence of this imbalance may result in a relative resistance of B lymphocytes to regulatory signals such as IFN-gamma, as is observed in adult patients with protracted disease.

Recently, both IFN-gamma and IFN-alpha had been used in the treatment of patients with severe AD (13,14,15) or hyper-IgE syndrome (16). In a significant proportion of them, a reduction of the serum IgE level and of disease intensity was achieved. For the treatment of patients with AD, IFN-gamma seems to be superior to IFN-alpha.

Our findings, demonstrating the preserved susceptibility of B cells to IFN-gamma in most children with AD and a proportion of adult patients, support the proposal to use IFN-gamma in AD therapy. The in vitro assay may be of predictive value for therapeutic success and can be helpful in selecting responsive patients for an optimal schedule of IFN-gamma therapy.

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