

Interferon therapy for atopic dermatitis reduces basophil histamine release, but does not reduce serum IgE or eosinophilic proteins

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Nine adult patients suffering from severe atopic dermatitis (AD) and increased total serum IgE were treated with recombinant human interferon-alpha 2A (Roferon-A^R; IFN- α -2A) 3×10^6 units daily for 21 d to study the effect upon serum IgE, basophil histamine release (HR), eosinophil-derived proteins in serum, and clinical symptoms. The skin disease was so severe that all patients needed topical treatment with glucocorticosteroid cream. Changes were not observed in total serum IgE or in eosinophil-derived proteins, the latter being increased in six of nine patients. A significant reduction in basophil HR was found in six of nine patients after anti-IgE and concanavalin A (Con A) stimulation, but not after A23187 stimulation. The clinical skin score was reduced in eight of nine patients at the end of therapy, but disease activity returned to pretreatment levels within 3 weeks despite continued topical treatment. rIFN- α -2A was well tolerated with few clinical side-effects, and all patients completed the study. The short-term therapy using IFN- α -2A neither brought a sustained clinical remission nor reduced total serum IgE or eosinophil-derived proteins. However, a significant reduction in IgE-receptor-mediated basophil HR was observed in six of nine patients.

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Severe atopic dermatitis (AD) is a chronic, disabling disease for which no cure exists. A significant correlation is observed between disease activity and total serum IgE, reflecting the abnormalities of T- and B-lymphocyte function (30). A recent case report showed that interferon-alpha (IFN- α) treatment reduced serum IgE by 50% together with an improvement of the eczema (28). Interferon-gamma (IFN- γ) is an important downregulator of interleukin-4 (IL-4)-driven IgE synthesis *in vitro* (14, 18), and both IFN- α and IFN- γ have now been used in a number of AD patients (2, 9, 16, 24, 32, 34). Our study of IFN- α 3 million units (MIU) twice weekly for 6 weeks could not demonstrate a reduction of circulating IgE levels (32), although a subsequent use of 3 MIU of IFN- α daily for 10 d led to reduction of serum IgE in three of four patients (unpublished data). This indicates the possibility of augmenting the therapeutic benefit by increasing the dosage of IFN- α .

This paper presents the results of a phase I study

in which nine adult AD patients with increased serum IgE were given subcutaneous injections of IFN- α 3 MIU daily for 3 weeks. The effect was assessed by a clinical skin score system and measurements of serum IgE levels. In addition, *in vitro* assays for basophil histamine release (HR) were performed, and serum values of the eosinophil-derived granule proteins, ECP and EPX/EDN, were used as a measure of eosinophil activity.

Material and methods

Study population

Nine adult patients entered the study after informed consent, four women and five men aged 18–39 years, median age 24 years. Four patients had rhinitis and one had asthma. None received therapy for their respiratory allergies during the study. All patients fulfilled the criteria for AD defined by Hanifin & Rajka (10). Their AD was present since childhood. All had active disease at the time of IFN- α therapy.

None had received PUVA or systemic prednisone within 3 months before entering the study. All patients used topical corticosteroids, which were continued during the study.

The study was approved by the ethical committee of Aarhus county.

Treatment regimen

Recombinant IFN- α -2A (Roferon-A[®], F. Hoffmann La-Roche, Basel, Switzerland) (hereafter called IFN- α) was administered subcutaneously as 3 MIU daily for 21 d. After receiving the initial injections in the outpatient clinic, patients continued treatment by self-administration. Empty vials were returned by all patients; the compliance was 100%. The patients were also given topical diflorason acetate (Soriflor[®]) for application once daily. Patients were offered concomitant paracetamol treatment to suppress possible flu-like effects from IFN- α .

Scoring of clinical observations

The patients were seen weekly for clinical examination by the same dermatologist (KTP) and for blood tests. An index score of disease severity was used by combining the severity rating for erythema, desquamation, infiltration, and pruritus as none, mild, moderate, or severe (0–3) with the extent of disease (0–6) in head, trunk, upper limbs, and lower limbs, giving a maximum score of 72 points (8).

Blood tests

The following tests were performed weekly for 4 weeks: hemoglobin, leukocytes, thrombocytes, aspartate aminotransferase (ASAT), and se-creatinine. Total IgE was determined weekly for 4 weeks and then every 2 weeks up to 12 weeks. Furthermore, basophil HR, and measurements of the eosinophil-derived proteins were also performed.

Basophil histamine release

Basophil HR was performed on washed blood cells with a standardized panel of secretagogues and a microfiber-based HR assay, as previously described (27, 38). Briefly, two 10-ml vials of blood were obtained by venipuncture and anticoagulated with Li-heparin 15 IU/ml or Na-K-EDTA 5 mM. The blood was stored for 16–20 h at 4°C, after which each vial was divided into two aliquots of 5 ml and centrifuged at 2755 g to isolate plasma. The blood cells were divided, resuspended, and washed in PIPES-buffered Na-K-acetate (PA) at an osmolarity of either 300 mOsm/kg H₂O or 500 mOsm/kg H₂O. Thus, four aliquots of blood cells were isolated, each

differing in type of anticoagulant used and osmolarity of the PA buffer.

Cells were distributed in 96-well, microfiber-containing microtiter-plates (Reference Laboratory, Copenhagen, Denmark) containing the standard panel of stimuli and histamine standards. Each well contained 50 μ l of PA buffer, 50 μ l of cell suspension, and 20 μ l of secretagogue, or histamine standard dissolved in PA buffer. The following secretagogues were used: rabbit-antihuman-F_c ϵ -antibody (anti-IgE; Behringwerke, Marburg, Germany) at final concentrations of 0.04, 0.4, 4, 20, 40, 200, and 400 U/ml; concanavalin A (Con A) (Pharmacia, Uppsala, Sweden) at 3.3, 16.6, 83.2, and 416 μ g/ml; *N*-formyl-methionyl-leucyl-phenylalanine (FMLP; Sigma, St Louis, MO, USA) at 10⁻⁸, 10⁻⁶, and 10⁻⁴ M; the calcium ionophore A23187 (Sigma) at 0.5, 1.0, and 2.0 μ M; and monoclonal mouse-antihuman-IgG4 (Oxoid, Basingstoke, UK) in 10-fold dilution steps of 1:100 to 1:10 000 of the stock solution.

All concentrations of secretagogues were used in triplicate, and each concentration range was preceded by blanks in triplicate containing only cells and buffer. A further three wells contained only PA buffer, and were used to estimate the spontaneous histamine release.

Fluorometric analysis with *o*-phthaldialdehyde was performed as described (27).

Histamine release

HR was quantitated by establishing a linear regression curve from histamine standards, 0, 20, 40, 60, and 80 ng/ml being added to wells containing washed blood cells. Spontaneous HR was estimated by subtracting the fluorescence in wells containing only PA buffer from the fluorescence in wells containing cells and PA buffer. On the basis of the variability of fluorescence measurements from the wells containing only washed blood cells and PA buffer, an HR >4 ng/ml blood was considered to be detectable, i.e., significantly different from background.

Measurement of cellular histamine content in lysed blood

Two hundred microliters of washed whole blood cells from each experiment was frozen at -20°C. After thawing, the lysed cells were diluted 10 times in 0.9% NaCl containing 0.5% CTAB (cetyltrimethylammonium bromide; Aldrich, Milwaukee, WI, USA), and heated to 85°C for 15 min. The denatured proteins were sedimented by centrifugation, and the supernatant was diluted again (1:4) in 0.9% NaCl for determination of total cellular histamine content.

Measurement of eosinophil-derived proteins

Two secretory products of the eosinophil granulocyte, eosinophil cationic protein (ECP) and eosinophil protein X/eosinophil-derived neurotoxin (EPX) were measured in serum by newly developed ELISA methods (21–23). The normal range for ECP is 2.3–15.0 ng/ml, mean 6.0 ng/ml; for EPX, 7.8–40.0 ng/ml, mean 17.8 ng/ml. Furthermore, a commercial radioimmunoassay (RIA) for ECP was used to measure ECP in serum according to the manufacturer's instructions (Pharmacia, Uppsala, Sweden). Serum for these measurements was obtained from blood allowed to clot at room temperature for 1 h before centrifugation and collection of serum.

External control populations and validation of assay reproducibility

For determination of the interassay variation, duplicate assays were performed on separate microtiter plates, four concentrations of anti-IgE being used as the stimulus (38). In 88 blood samples prepared in 500 mOsm/kg H₂O buffer, the median numeric difference between duplicate measurements was 2 ng/ml blood (quartiles 0–4 ng/ml). In 300 mOsm buffer, the corresponding values were 2 ng/ml blood (median) (quartiles 1–5 ng/ml; $n = 77$).

For assessment of the spontaneous week-to-week variation in basophil HR, a group of nine volunteers had been tested six times at weekly intervals before the commencement of this study. Blood samples were anticoagulated with EDTA and washed in 500 mOsm/kg H₂O PA buffer, and anti-IgE was used as secretagogue.

Statistics

Nonparametric statistical methods were employed: Wilcoxon's test for paired analysis and Mann-Whitney's U-test for comparison of two nonpaired groups, as well as Kruskal-Wallis' one-way and Friedman's two-way analysis of variance. Spearman's method based on ranks was used for correlation analysis.

Results*Clinical observations*

Eight of nine patients showed reduced severity and extent of disease during the treatment period (Fig. 1). The median score index showed a clear reduction during treatment from 4.9 to 1.6, although this was not statistically significant because of the wide range in the clinical indices. The median score index increased to above pretreatment levels (6.1) 3 weeks

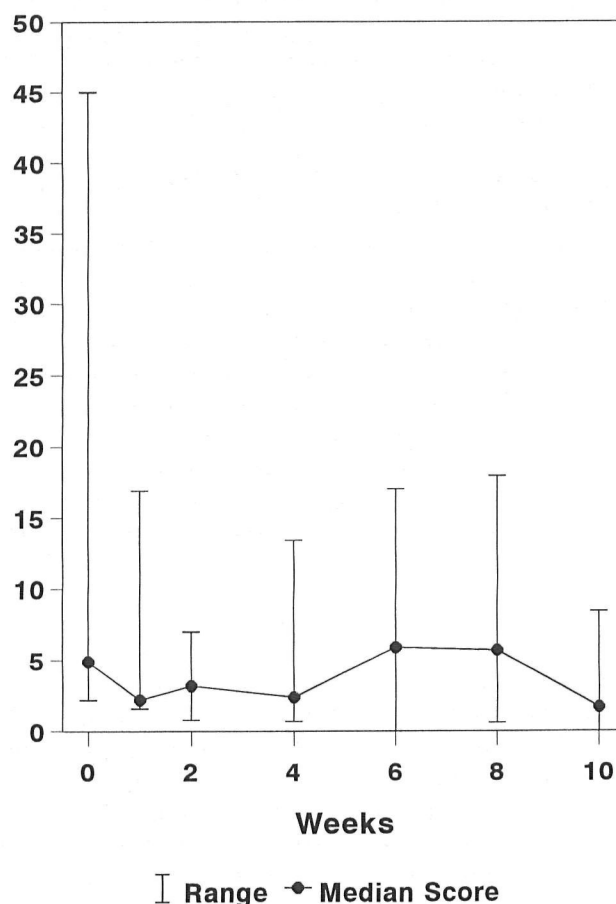
Skin Score Index

Fig. 1. Median clinical score before, during, and after IFN- α -2A therapy, 3×10^6 daily for 21 d.

after stopping IFN- α . Four weeks later, the median score was again 1.4. The amounts of steroid cream used were not measured precisely, but, on average, less than 30 g per week per patient was used during the treatment and follow-up period.

All patients tolerated daily IFN- α well. It caused only mild, flu-like symptoms, which subsided within the first week of treatment. Dosage reductions were not necessary and the patients took paracetamol only during the initial days.

The long-term effect of the therapy has also been evaluated. One year after therapy, all patients are doing well with intermittent use of topical steroid. None are seen regularly because their overall disease activity is reduced.

Biochemistry

All monitored blood indicators stayed within the normal range, and none changed significantly during the treatment period (results not shown). The number of circulating eosinophils or basophils was not recorded.

Total serum IgE. Total serum IgE was 4126 units/l (median value) at the start of the study with a range of 650–15315 units/l. This value did not change during treatment or during the follow-up period (Table 1; Fig. 2).

Basophil histamine release. The results from basophil HR are summarized in Table 1. The median HR induced by anti-IgE and ConA was significantly reduced during IFN treatment ($P < 0.05$; Friedman's two-way analysis of variance). Six out of nine patients showed a dramatic reduction in IgE-mediated HR (Fig. 3A, B), while two patients showed no significant change, and one patient had a barely detectable HR throughout the study period (Fig. 3C). Maximum HR induced by ConA stimulation correlated well with the HR seen with anti-IgE ($r_s = 0.87$; $P < 0.01$). Neither the sensitivity of the cells to anti-IgE or ConA, expressed as the EC_{50} , nor the shape of the dose-response curve changed during the IFN- α treatment (data not shown).

Basophil HR did not correlate with the clinical score of the patients, and there was no significant correlation between the magnitude of clinical changes and changes in IgE-receptor-mediated basophil HR. The HR induced by calcium ionophore A23187 did not change significantly during the study, and was less than anti-IgE- and ConA-induced release (Table 1). The maximum HR after anti-IgE, ConA, and A23187 stimulation was significantly higher in blood anticoagulated with EDTA than in blood from the same venipuncture anticoagulated with heparin ($P < 0.05$; paired analysis). However, the actual differences were small (medians 2, 3.25, and 2 ng histamine/ml blood, respectively). The histamine

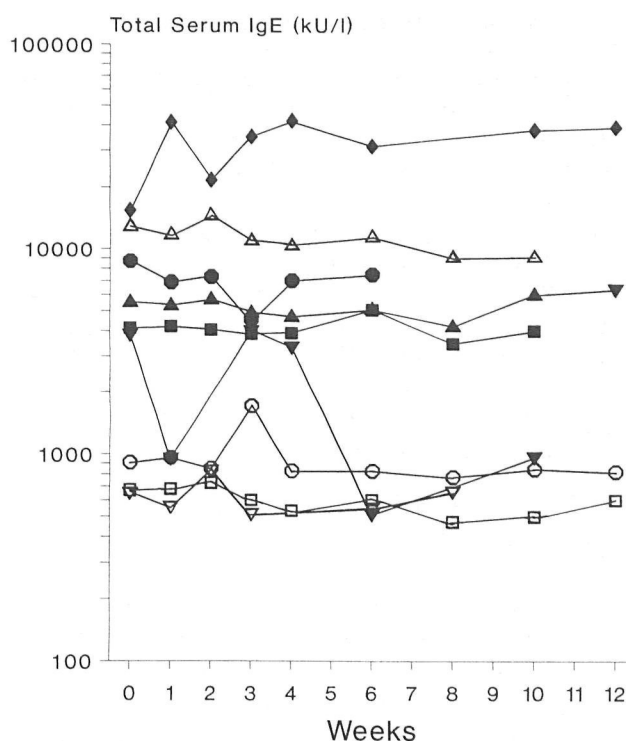


Fig. 2. Total serum IgE in nine patients before, during and after treatment with IFN- α -2A, 3×10^6 units daily for 21 d.

content of lysed blood cells did not change significantly during the treatment with IFN- α in any patient (data not shown).

Spontaneous biologic variation of HR. The median anti-IgE-induced HR in the nine atopic patients was compared with anti-IgE-induced HR in nine healthy

Table 1. Effects of IFN- α -2A on immunologic variables

		Pretreatment		During treatment		Post-treatment	
Total s-IgE (U/ml):	Median Range	4126 (650–15315)		4045 (535–35033)		3890 (513–36685)	
Maximum HR (ng/ml blood)							
Spontaneous*	(300 mOsm)	≤ 4	(1–5.5)	≤ 4	(1–4.5)	≤ 4	(2.5–6)
	(500 mOsm)	8	(1–14.5)	6	(5–11)	6.5	(3–13)
Anti-IgE*	(300 mOsm)	14	(5.5–35)	10	(3.5–13.5)	13	(4–38)
	(500 mOsm)	24	(10.5–42)	10.5	(3–21)	36	(7–41)
Concanavalin A*	(300 mOsm)	11	(3.5–38)	7.5	(2–9)	15	(3–37)
	(500 mOsm)	23	(5.5–41)	9.5	(4.5–16.5)	23	(4–48)
A 23187*	(300 mOsm)	10	(2–15.5)	8	(4–15)	10.5	(8–15.5)
	(500 mOsm)	6.5	(2–28)	10.5	(4.5–14.5)	12	(7–18)
s-ECP (ng/ml)		22	(6.5–40)	16.5	(3–44)	22	(6–66.5)
s-EPX/EDN (ng/ml)		36	(14–70)	34	(11.5–75)	41	(11–96)

* Comprises measurements from both EDTA and heparin-anticoagulated blood.

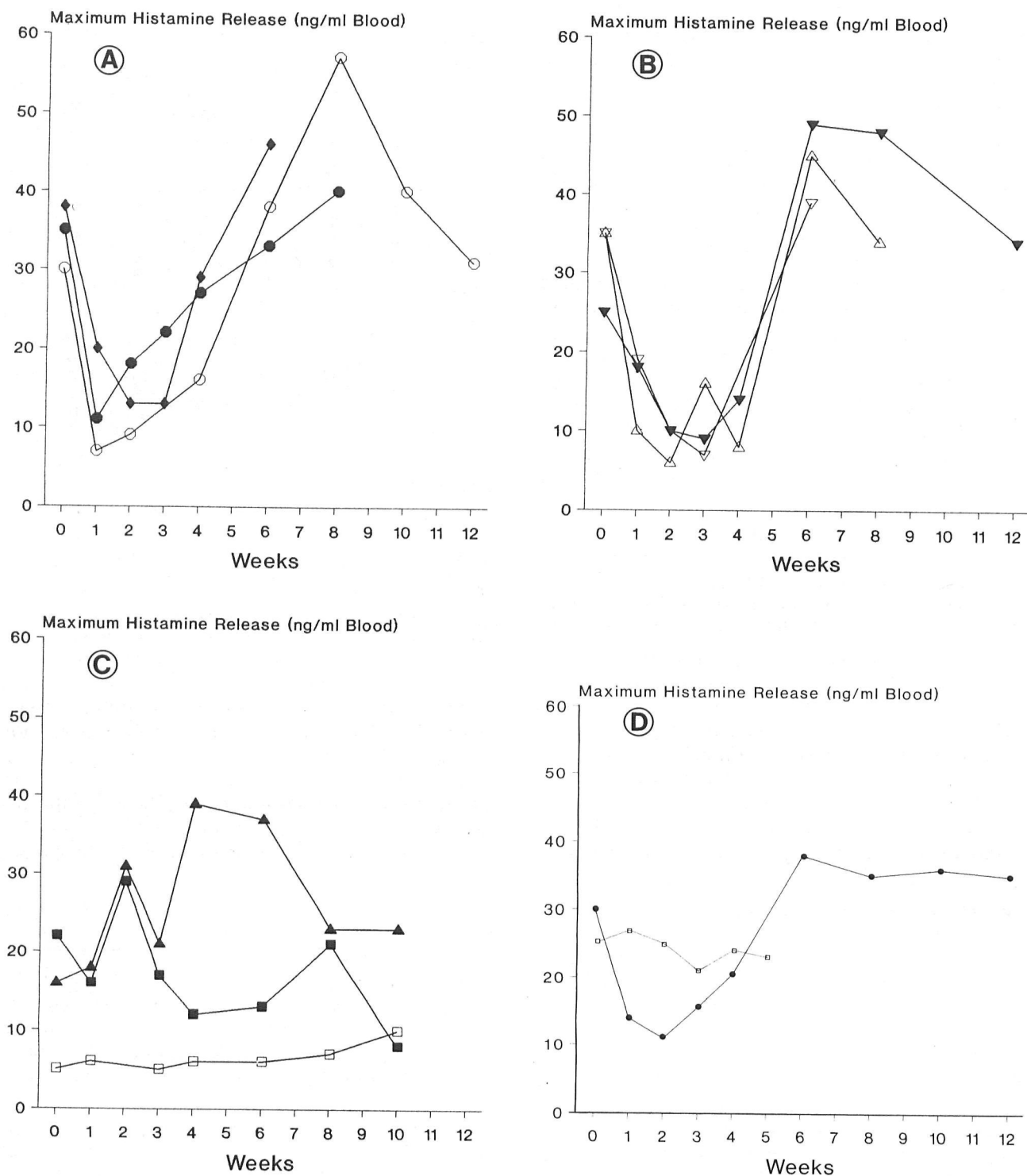


Fig. 3. A and B) Basophil HR after anti-IgE stimulation before, during, and after IFN- α therapy, 3×10^6 units daily for 21 d. Six patients showed significant reduction in HR. C) Two patients did not show change, and one patient had minimal HR after anti-IgE stimulation throughout study. D) Median value of anti-IgE-induced HR in nine patients (dark circles) compared with median value in nine healthy control persons (open circles) without any medication during observation period of 5 weeks.

controls (Fig. 3D). It is seen that anti-IgE HR in healthy persons did not fluctuate to any significant degree.

Basophil HR induced by anti-IgG4. We observed HR in four out of nine patients using anti-IgG4. The HR was dose-dependent and showed a bell-shaped con-

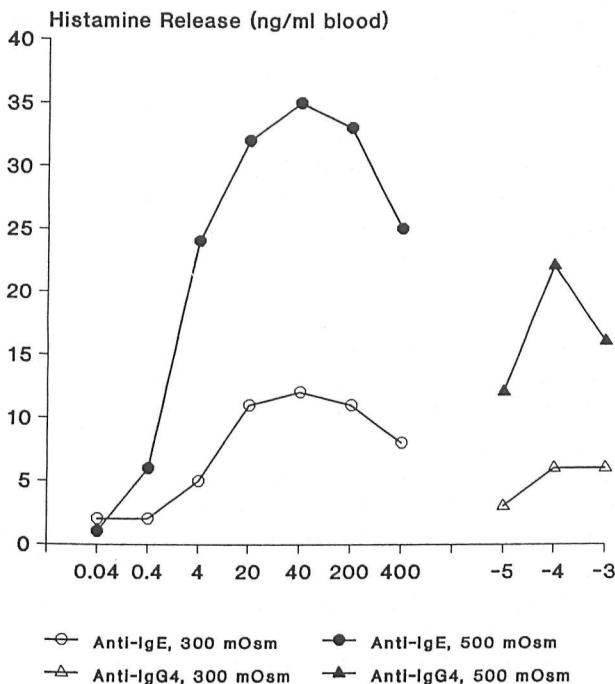


Fig. 4. Anti-IgG4 induced HR in 4/9 patients in study. Representative experiment is shown; stimuli were anti-IgE (circles) and anti-IgG4 (triangles) with either normo-osmolar buffer (open circles or triangles) or hyperosmolar buffer (closed circles or triangles). Abscissa depicts concentrations of antibody in units/ml for anti-IgE and in $\log_{10}$ -dilutions of stock for anti-IgG4.

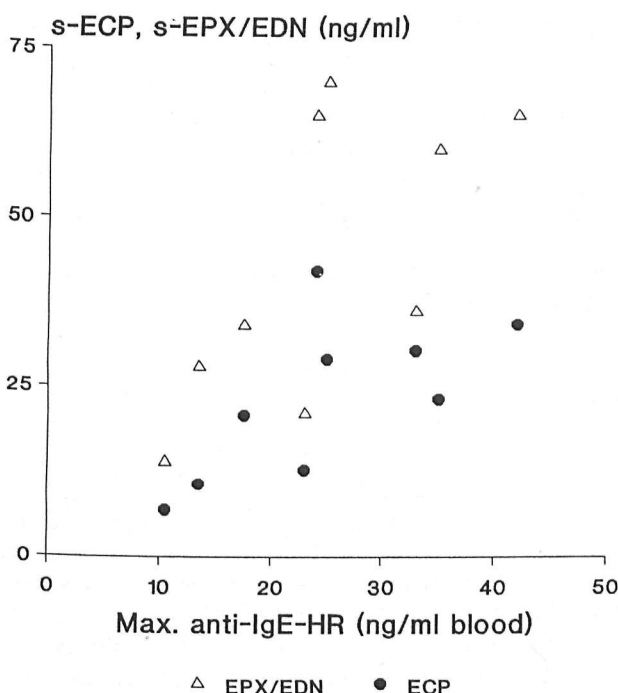


Fig. 5. Relation between anti-IgE-induced HR from washed blood in hypertonic buffer before start of IFN- α 2A therapy and serum levels of eosinophil-derived proteins ECP and EPX/EDN. Positive correlation between anti-IgE-induced HR and s-ECP and s-EPX is noted (Spearman's $\rho = 0.87$; $P < 0.05$).

centration response (Fig. 4). Furthermore, the anti-IgG4-induced HR showed a significant potentiation when cells were tested in hyperosmolar rather than normo-osmolar buffer. The anti-IgG4-induced HR showed large fluctuations during the observation period with no correlation to the treatment with IFN- α .

Total serum IgE content and serum ECP (s-ECP). The median s-ECP and s-EPX values before, during, and after treatment are shown in Table 1. Correlations between s-ECP and s-EPX and the maximum HR induced by anti-IgE before IFN- α treatment were significant (Fig. 5) ($r_s = 0.77$; $P < 0.01$). However, s-ECP and s-EPX did not change significantly during the treatment with IFN- α , although a trend towards increasing values was noted after stopping therapy. There was no correlation between anti-IgG4-induced HR and s-ECP or s-EPX. Measurement of s-ECP by ELISA or RIA (Pharmacia) yielded almost identical results ($r_s = 0.96$); the median difference between parallel measurements with the two methods was only 2.5 ng/ml.

Discussion

We are aware of seven published studies on the effects of IFN administration to patients with AD (2, 9, 16, 24, 28, 32, 34). None have observed a significant reduction in total serum IgE, but one has shown that blood cells *in vitro* release less spontaneous IgE during the therapy in five of six patients (2, 18). This study confirms the lack of an effect on total serum IgE during daily IFN- α administration of 3 MIU for 21 d.

We found a significant reduction of IgE-receptor-mediated basophil HR in six of nine patients during IFN- α treatment, followed by an immediate or slightly delayed return to the initial level after withdrawal of IFN- α (Fig. 3A–C). Extensive control experiments using our HR assay system have verified that the reduction of basophil HR was significantly larger than the interassay variation. Furthermore, the observed changes were far beyond the spontaneous fluctuations in HR observed in nine adult volunteers without medication (Fig. 3D), or in a previous study during treatment of atopic patients with herbal extracts (29). One patient had a very low HR and two patients had a high HR, but did not show any change of IgE-mediated HR during the IFN treatment (Fig. 3C, filled symbols). In contrast to the remaining seven patients, they were found to have antibodies against IFN in their serum (Roos et al., in preparation).

The reduced HR could be caused either by a decreased number of circulating basophils during IFN- α therapy, or by degranulation of the basophils. Circulating basophil counts are reported to be ele-

vated, but relatively constant over long observation periods in atopic patients (6, 20). We did not count basophils in our patients, but there was no change in the total histamine content of cell lysate during IFN- α therapy.

Some of our patients expressed a clear reduction in pruritus during IFN- α treatment, although this variable alone did not correlate with the observed changes in basophil HR. The clinical effect may be due to the simultaneous topical treatment with a potent steroid, but a number of patients showed a clear worsening of their AD immediately after the IFN- α treatment was stopped even though they continued to use topical steroid freely.

It is well known that steroids can inhibit IgE-receptor-mediated basophil HR *in vitro* (25). However, serum cortisol levels did not change significantly during topical application of the same topical steroid (Soriflor) in psoriasis (K. Thestrup-Pedersen; unpublished), although dosages were far higher than those used in the present study. Similarly, the AD patients given herbal drugs also used topical steroids, which did not influence basophil HR (29). We therefore conclude that the reduced basophil HR during treatment with IFN- α represents a biologically relevant effect of this cytokine *in vivo*.

The mechanisms of action of IFN- α on the basophil HR are not yet known. Virus-induced IFN in culture supernatants has been reported to increase basophil HR (3, 11). In contrast, a recent report showed that 24-h incubation of highly purified rat peritoneal mast cells with recombinant IFN- α led to an inhibition of IgE-mediated HR, whereas HR induced by non-IgE-mediated stimuli (compound 48/80 and substance P) was not affected (31). Extended incubation periods with IFN- α were necessary to show an effect on HR in the rat mast cells. We have performed preincubation with IFN- α and washed blood cells from atopic patients for 1 h and found no effect on IgE-mediated HR (data not shown).

Bone marrow cells of basophil or mast cell lineage have recently been shown to respond to anti-IgE stimulation by producing large amounts of IL-4 (19). IL-4 is a potent stimulator of IgE synthesis, antagonized in most *in vitro* B-cell systems by IFN- γ or IFN- α (4, 5, 14, 18, 33, 35, 37). However, IFN- γ is not effective in all assay systems; in a recent clinical trial, IFN- γ failed to reduce circulating IgE levels (2). Thus, it is still an open question whether an ongoing IgE production can be modulated *in vivo* after the B-cell isotype switch has already taken place (7).

The level of ECP in serum is elevated in patients with AD and correlates with disease severity (12). The serum levels of ECP and EPX were strongly correlated to each other in our study. Each patient

had a characteristic profile, which did not change during treatment. The observed correlation between anti-IgE-induced HR from the basophils and s-ECP and s-EPX (Fig. 5) has not been reported previously. It agrees with a recent observation in our laboratory of an increased IgE-mediated HR in blood from patients with eosinophilia caused by parasitic infection (39).

Anti-IgE autoantibodies have been demonstrated in allergic disease, and have been implicated as mediators of anti-IgG-mediated basophil HR (15). We consistently observed HR in four patients after anti-IgG4 stimulation which was not correlated with s-ECP or s-EPX levels. Interestingly, the anti-IgG4-induced HR was clearly potentiated by the hypertonic solution, in analogy with anti-IgE- and ConA-induced HR, but in contrast to A23187-induced HR (38). This suggests that the anti-IgG4-induced signal shares signal transduction characteristics with the IgE-receptor-mediated pathway. Indeed, the β -subunit of the Fc ϵ R $_1$ on mast cells has been shown to combine with a low-affinity IgG-receptor (Fc γ IIIa, CD16) (13), although on basophils only the Fc γ RII (CDw32) could be demonstrated by immunofluorescent staining (1). However, our data are also compatible with the presence of IgG4 anti-IgE autoantibodies complexed with basophil-bound IgE (15).

A recent multicenter study using IFN- γ in AD has confirmed a significant clinical effect upon disease activity, whereas there was no reduction in total serum IgE (Kevin Cooper, personal communication). There are, however, major differences between IFN- α and IFN- γ (26). A recent study in patients with renal carcinoma showed that IFN- α increased MHC class I antigen expression on cells from tumor tissue and increased CD4+ cells and cells carrying T-cell receptors among tumor-infiltrating lymphocytes, whereas IFN- γ upregulated MHC class II and increased CD8+ and TCR cells in the same compartments (17). Similarly, these IFNs have a different effect on Fc-receptor expression on monocytes (36).

In conclusion, treatment of AD patients with daily injections of 3 MIU of IFN- α during 21 d was well tolerated, and disease activity was reduced during therapy. IFN- α treatment significantly decreased the IgE-mediated basophil HR. No effect could be shown on serum IgE levels or the eosinophil secretory products s-ECP and s-EPX. The mechanisms of action of IFN- α on the basophil granulocyte remain to be shown. Therefore, we need further carefully designed studies using biologic response modifiers in a disorder involving disturbances of major parts of the immune inflammation.

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