

Antigen presenting cell-independent cytokine and spontaneous in vitro IgE production in patients with atopic dermatitis: Increased interferon- γ production and lack of effects of in vivo low-dose interferon- γ treatment

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Atopic dermatitis is characterized by elevated serum IgE concentrations and dysregulation of T-lymphocyte function. To examine the pattern of cytokine production associated with elevated IgE levels, phorbol ester plus ionomycin-stimulated production of interleukin (IL)-4, IL-5, and interferon- γ (IFN- γ) by blood mononuclear cells from 16 patients with atopic dermatitis was compared with that of 18 healthy subjects. Spontaneous in vitro IgE production was also studied longitudinally in patients receiving placebo or daily treatment with 0.05 mg/m² IFN- γ . Spontaneous in vitro IgE production and mitogen-driven IL-4 and IFN- γ synthesis did not differ when patients were receiving interferon treatment compared with no treatment. Furthermore, ionomycin plus phorbol ester-stimulated mononuclear cells from patients with atopic dermatitis produced less IL-4 and more IFN- γ than did cells from healthy subjects. IL-5 production by cells from patients with atopic dermatitis did not differ from that of cells from healthy subjects. The ratio of IL-4 to IFN- γ produced in vitro was significantly lower ($p = 0.04$) in the cells of patients with atopic dermatitis (0.9) as compared with those of healthy subjects (2.7). The findings suggest that when circulating T cells are stimulated under antigen presenting cell-independent conditions, atopic dermatitis is not characterized by the shift in the reciprocal relationship between IL-4 and IFN- γ production, which has been postulated to explain the pathogenesis of IgE elevation and the therapeutic action of IFN- γ in patients with atopic dermatitis. (J ALLERGY CLIN IMMUNOL 1995; 96:84-91.)

Key words: Atopic dermatitis, IgE, IL-4, IL-5, interferon- γ

Atopic dermatitis is characterized by cutaneous T-lymphocyte activation, hyperfunctional Langerhans' cells, and increased systemic production of

Abbreviations used

IFN- γ : Interferon- γ

IL: Interleukin

PBMCs: Peripheral blood mononuclear cells

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IgE.¹ A proportion of this IgE is specific for antigens, which may act as exacerbating factors in this disease.²⁻¹¹ These antigen-induced exacerbations may be IgE-mediated by either Langerhans' cell antigen presentation to T cells⁹ or cutaneous mast cell mediator release.¹² T-lymphocyte regulatory factors such as interleukin-4 (IL-4) and interferon- γ (IFN- γ) reciprocally induce¹³⁻¹⁶ and suppress¹⁷⁻²⁰ human in vitro IgE production. It has been postulated that dysregu-

TABLE I. Subject characteristics

Subject No.	Age (yr)	Sex	Systemic medications	Pretreatment serum IgE concentrations (IU/ml)	Pretreatment spontaneous in vitro IgE (pg/ml)
1	48	M	None	5,633	ND
2	19	F	None	2,382	2,516
3	35	F	None	3,651	2,337
4	48	F	Terfenadine	1,783	457
5	20	F	None	14	4,235
6	47	F	None	1,496	1,842
7	23	F	None	92	767
8	51	M	None	6,045	ND
9	37	M	None	3,351	ND
10	57	F	Hydrochlorothiazide	3,124	443
11	32	M	None	9,222	1,612
12	29	F	Hydroxyzine	11,000	3,463
13	34	M	None	>11,000	35,646
14	32	M	None	11,000	29,254
15	31	F	Hydroxyzine	3,135	ND
16	53	M	Terbutaline + H ₁ -blocker*	714	2,306

ND, Not done.

*Unspecified H₁-antihistamine.

lation in the production of these cytokines contributes to the pathogenesis of atopic dermatitis. Indeed, peripheral blood mononuclear cells (PBMCs) from patients with atopic dermatitis have been shown to manifest decreased production of IFN- γ ^{21, 22} and enhanced responsiveness to IL-4.²³ Lesional skin of patients with atopic dermatitis also contains lymphocytes that produce relatively increased amounts of IL-4, IL-5, and IL-10, but little IFN- γ .²⁴⁻²⁷ These observations suggest a T_{H2}-type cytokine response in antigen-sensitized T cells.

To suppress a T_{H2}-like response in a human disease, IFN- γ was administered to patients with atopic dermatitis. Spontaneous in vitro IgE production was inhibited by in vivo IFN- γ treatments in 10 of 14 patients with atopic dermatitis, although total serum IgE concentrations were not affected.²⁸

Because no change in serum IgE, despite clinical improvement and decreasing eosinophil counts, has been noted in patients with atopic dermatitis treated with IFN- γ , the mechanism of action of IFN- γ in atopic dermatitis remains unknown. We report the effects of in vivo administration of IFN- γ on spontaneous in vitro IgE production, as well as in vitro mitogen-induced production of IL-4, IL-5, and IFN- γ by mononuclear cells from patients with atopic dermatitis.

METHODS

Patient population

Sixteen patients (7 men and 9 women) with a median age of 34 years (range, 19 to 57 years) with atopic dermatitis were studied. The diagnosis of atopic dermatitis was made with the criteria of Hanifin and Rajka.²⁹ Characteristics of the subjects, whose clinical responses have been published elsewhere,³⁰ are presented in Table I.

Eighteen healthy control subjects were studied for comparative purposes. Seven of the eighteen subjects were laboratory workers. The remaining subjects were blood bank donors.

This study was approved by the Institutional Review Board of the University of Michigan Medical Center. Informed consent was obtained from each subject.

Administration of IFN- γ

Active treatment with recombinant IFN- γ or placebo was administered in a double-blind trial. IFN- γ 0.05 mg/m² or placebo was administered subcutaneously daily for 12 weeks. All of the subjects initially randomized to receive placebo were subsequently treated with open-label IFN- γ . Blood samples for in vitro studies were obtained before treatment with IFN- γ and at various intervals after initiation of treatment.

In vitro IgE production

Spontaneous in vitro IgE production was studied in 12 of the 16 subjects. Eight subjects initially received placebo injections, and four initially received injections of IFN- γ . All of the patients receiving placebo also had in

vitro IgE measurements after crossover to IFN- γ treatments during the unblinded phase of the study. The duration of this unblinded phase of treatment was variable and lasted up to 9 months in some subjects. PBMCs were obtained from preservative-free heparinized blood by Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) density gradient sedimentation. Cultures for the measurement of supernatant IgE production were performed in RPMI-1640 medium (Gibco, Grand Island, N.Y.) with 10% fetal calf serum, 80 μ g/ml gentamicin, and 25 mmol/L N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid. PBMCs (10^6 /ml) were placed in flat-bottomed microtiter wells (220 μ l/well). Replicate cultures were done in up to 12 wells, and the supernatants were pooled into quadruplicate samples before assays were performed. Cultures were incubated at 37° C in 5% CO₂ atmosphere for 9 days with and without cycloheximide (100 μ g/ml). At the end of the culture period, cells were centrifuged at 2500 rpm for 10 minutes, and the supernatants were removed and stored at -70° C.

In vitro cytokine production

Production of IL-4 and IFN- γ from stimulated cells of untreated patients with atopic dermatitis was compared with that from cells of healthy subjects and with that from cells of patients treated with IFN- γ . IL-5 production from cells of patients with atopic dermatitis and healthy subjects was also compared. Most but not all patients were studied.

Mixed PBMCs were obtained and suspended in medium as described above except at a concentration of 2×10^6 cells/ml. Cultures were performed in duplicate, triplicate, or quadruplicate, depending on the number of cells available, and cultured with and without ionomycin 1 μ g/ml and phorbol myristate acetate 50 ng/ml for 48 hours. Supernatants were then removed and stored at -70° C until assays were performed.

ELISAs for IgE, IL-4, IL-5, and IFN- γ

IgE in culture supernatants was measured with an avidin-biotin amplified micro ELISA exactly as previously described.³¹ Biotinylated anti-human IgE monoclonal antibody (4.15, from A. Saxon, University of California, Los Angeles) was used in the IgE ELISA. ELISAs for IL-4, IL-5, and IFN- γ were performed with antibodies and methods as previously described.³² Nitroiodophenyl-conjugated rabbit anti-human IL-4 antibody 25D2 and two rat anti-human monoclonal antibodies that specifically recognize IL-5 were used in the IL-4 and IL-5 ELISAs, respectively (kindly provided by Dr. Jon Abrams of DNAX Co., Palo Alto, Calif.).

Statistical analysis

Data were analyzed by two-tailed Student's *t* test for unpaired samples, for paired samples, and for regression analysis. Fisher's exact test was used to analyze the difference between proportions.

RESULTS

Effect of IFN- γ treatment on spontaneous IgE production

The clinical responses of these patients to initial treatment with IFN- γ have been reported elsewhere.³⁰ Serum IgE levels in these patients ranged from 14 to greater than 11,000 IU/ml before treatment (Table I). Spontaneous in vitro IgE production was measured before initiation of IFN- γ or placebo treatments. PBMCs from 15 to 16 subjects demonstrated measurable IgE production as compared with cycloheximide-treated negative control cultures. There was a trend toward a positive correlation between serum IgE and spontaneous in vitro IgE production in those patients in whom both measurements were made ($r = 0.55$, $p = 0.059$, $n = 12$) (Table I).

During the double-blind phase of the protocol, four subjects received IFN- γ , and eight subjects received placebo (Table II). All four of the subjects who initially received active treatment (nos. 5, 6, 13, and 16) showed a decrease in spontaneous in vitro IgE production ($p < 0.01$, Student's *t* test). However, cells from three of eight subjects treated with placebo (nos. 11, 12, and 14) also demonstrated decreased spontaneous in vitro IgE production. All of the subjects treated with placebo were subsequently treated with open-label IFN- γ . Only two of these subjects (nos. 3 and 7) demonstrated decreased in vitro IgE production while receiving IFN- γ when compared with baseline values. Overall, 12 treated subjects were evaluated, six of whom (nos. 3, 5, 6, 7, 13, and 16) showed a significantly decreased spontaneous in vitro IgE production compared with three of eight subjects receiving placebo (nos. 11, 12, and 14). The proportion of subjects treated with IFN- γ who had a decrease in spontaneous in vitro IgE (6 of 12) did not differ from the proportion of subjects treated with placebo (3 of 8) ($p = 0.31$, Fisher's exact test). However, among subjects with spontaneous IgE production of greater than 900 pg/ml at baseline, six of eight (nos. 3, 5, 6, 7, 13, and 16) demonstrated reduction of in vitro IgE production after treatment with IFN- γ .

Mitogen-driven IL-4, IL-5, and IFN- γ production in vitro in subjects with and without atopic dermatitis

IL-4 and IFN- γ production with and without IFN- γ treatment. Spontaneous IL-4 and IFN- γ production was very low or unmeasurable in all subjects. The arithmetic mean ($\pm$ SD) of mitogen-stimulated IL-4 production by PBMCs from pa-

TABLE II. Spontaneous *in vitro* IgE production from PBMCs

Subject No.	Baseline (pg/ml)	Follow-up placebo (pg/ml)	Active treatment (pg/ml)
2	2,516 ± 427	2,905 ± 375 (12 wk)	2,648 ± 106 (6 wk)
3	2,337 ± 149	2,592 ± 405 (12 wk)	383 ± 423 (25 wk)*
4	457 ± 426	375 ± 319 (7 wk)	53 ± 83 (30 wk)
5	4,235 ± 1,368	ND	1,575 ± 305 (7 wk)†
6	1,842 ± 428	ND	613 ± 123 (12 wk)‡
7	767 ± 271	967 ± 224 (12 wk)	213 ± 160 (30 wk)§
10	443 ± 308	0 (12 wk)	271 ± 244 (39 wk)
11	1,612 ± 546	237 ± 211 (12 wk)‡	1,212 ± 207 (12 wk)
12	3,463 ± 768	596 ± 236 (12 wk)*	4,368 ± 2,122 (12 wk)
13	35,646 ± 1,681	ND	4,707 ± 892 (26 wk)*
14	29,254 ± 2,095	1,483 ± 550 (12 wk)*	2,116 ± 220 (19 wk)
16	2,306 ± 555	ND	638 ± 413 (41 wk)‡

Values are expressed as means ± SD.

ND, Not done.

* $p < 0.001$, Student's t test, placebo versus baseline; active treatment versus baseline or last placebo value.

‡ $p < 0.005$.

† $p < 0.01$.

§ $p < 0.02$.

tients not receiving IFN- γ was 339 ± 428 pg/ml compared with 285 ± 440 pg/ml when the subjects were receiving IFN- γ treatment ($p = 0.775$, paired Student's t test). Mitogen-stimulated *in vitro* IFN- γ was equivalent in 11 subjects when receiving active treatment (mean = 631 ± 251 pg/ml) and when not receiving treatment (496 ± 361 pg/ml; $p = 0.2603$).

IL-4, IL-5, and IFN- γ production in patients with atopic dermatitis and healthy subjects. Mitogen-driven cytokine production by PBMCs from patients with atopic dermatitis was also compared with that of 18 healthy control subjects. Mean IL-4 production by PBMCs from patients with atopic dermatitis (341 ± 407 pg/ml) tended to be reduced relative to that of healthy control subjects (609 ± 616 pg/ml). Analysis of log-transformed data revealed that this change approached statistical significance ($p = 0.0501$) (Fig. 1, A). Mean IL-5 production of stimulated cells from patients with atopic dermatitis (300 ± 377 pg/ml) was not different from that of cells from healthy subjects (259 ± 241 pg/ml) (Fig. 1, B). There was a highly significant correlation between *in vitro* production of IL-4 and IL-5 ($r = 0.94$, $p = 0.0002$).

Mitogen-stimulated IFN- γ production in 13 untreated patients with atopic dermatitis (618 ± 234 pg/ml) was statistically equivalent to that of 11 healthy subjects (398 ± 343 pg/ml; $p = 0.0752$). If one outlier among the healthy subjects (1420 pg/ml) is omitted, the mean value for the remaining 10 subjects is 295 ± 56 pg/ml, which is significantly

lower than that of the patients with atopic dermatitis ($p = 0.0004$) (Fig. 1, C).

Reduced IL-4-IFN- γ ratio in atopic dermatitis. The ratio of mitogen-stimulated *in vitro* IL-4 to IFN- γ was derived for 13 patients with atopic dermatitis for whom data were available and compared with those derived for 11 healthy control subjects. The mean of the IL-4:IFN- γ ratio of the atopic patients (0.876 ± 1.22 [$n = 13$]) was more than three times lower than that of the healthy subjects (2.689 ± 2.67 [$n = 11$; $p = 0.04$]) (Fig. 2).

DISCUSSION

IgE-directed antigen reactivity is thought to be involved in the pathogenesis of atopic dermatitis.¹ Previously, it has been shown that 80% to 85% of patients with atopic dermatitis have elevated levels of serum IgE.³³ Furthermore, fluctuations in these levels are thought to reflect a parallel change in clinical severity.³⁰ Many investigations have focused on *in vivo* and *in vitro* modulation of IgE release from B cells. The induction of IgE synthesis requires B-cell activation by cognate or noncognate interactions.^{34,35} After activation, early B cells, in the presence of IL-4, undergo isotype switching to produce IgE antibody.³⁵ IFN- γ inhibits this response.¹⁷⁻²⁰ Recombinant IL-4-dependent IgE synthesis can be modulated by other cytokines. IL-4-induced IgE production by human mononuclear cells can be inhibited by IL-8, IL-10, IL-12, and transforming growth factor- β .³⁶⁻³⁹ IL-5 and IL-9 potentiate and

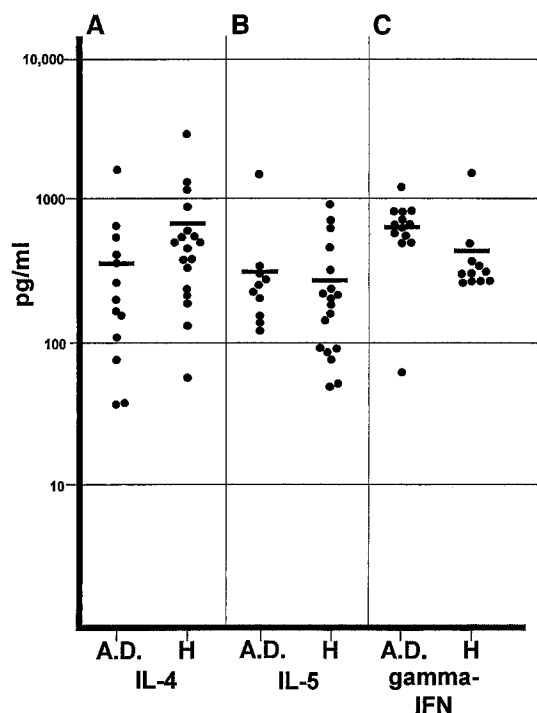


FIG. 1. Comparison of mitogen-stimulated in vitro cytokine production by PBMCs from patients with atopic dermatitis (A.D.) and healthy subjects (H). Results are expressed in picograms per milliliter. **A**, IL-4 production from patients with atopic dermatitis was reduced compared with that of healthy subjects ($p = 0.0501$). **B**, IL-5 production was equivalent in patients with atopic dermatitis and healthy subjects. **C**, IFN- γ production from patients with atopic dermatitis tended to be higher than that of healthy subjects. ($p = 0.075$) ($p = 0.004$ of outlier control subject omitted).

IL-13 induces IgE synthesis.⁴⁰⁻⁴² IL-4 also inhibits the synthesis of IFN- γ .⁴²⁻⁴⁴

Studies in mice and human beings have shown that cytokines that promote IgE production are produced by T-helper (T_H) cell subpopulations termed T_{H2} cells.⁴⁵ T_{H2} cells produce IL-4, IL-5, and IL-6. The addition of IL-4 to T lymphocytes results in the proliferation of T_{H2} cell clones, which can subsequently enhance IgE synthesis.⁴³ In contrast, T_{H1} cells produce IL-2 and IFN- γ , which suppress IgE production and inhibit expansion of T_{H2} clones.⁴⁶ Thus the ratio of secreted IL-4 to IFN- γ is important in determining the net synthesis of IgE. PBMCs from patients with atopic dermatitis have been reported to have a decreased activity of T_{H1} cells compared with T_{H2} cells.⁴⁷ Lesional skin from patients with atopic dermatitis contains T_{H2} cells,^{24, 25} many of which are specific for airborne antigens, such as dust mite and grass pollen.^{24, 48} Most such clones are T_{H2} cells, although T_{H1} clones can also be generated.²⁴ It is

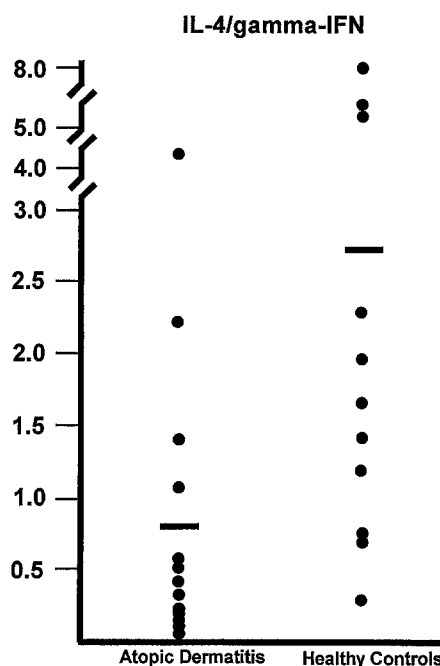


FIG. 2. Comparison of ratios of mitogen-stimulated in vitro production of IL-4 to that of IFN- γ in patients with atopic dermatitis and healthy subjects. PBMCs from patients with atopic dermatitis demonstrated a lower IL-4: IFN- γ ratio than cells from healthy subjects ($p = 0.04$).

now appreciated that chronic atopic dermatitis skin lesions contain T_{H1} cells, as compared with acute lesions.^{49, 50}

In this study we found a trend toward a correlation between serum IgE and spontaneous in vitro IgE production in patients with atopic dermatitis ($r = 0.55$, $p = .059$) (Table I). These results differ from those of much earlier atopic dermatitis studies, which did not show such a correlation.^{51, 52} Our results, however, are similar to those found recently in patients with filariasis and with hyperimmunoglobulin E-recurrent infection syndrome who have high serum IgE levels.^{53, 54} An explanation for the discrepancy between the early and current findings may lie in the use of polyclonal anti-IgE antibodies in the early in vitro assays.

This study demonstrated no difference in IL-4 levels in patients receiving or not receiving IFN- γ treatment. This has not been previously reported. There was also no difference in mitogen-driven IL-4 or IL-5 production by PBMCs between healthy control subjects and patients with atopic dermatitis. Similar results were obtained in a study by Del Prete et al.⁵⁵ Another study by Jujo et al.,²¹ however, reported markedly higher in vitro concanavalin A-stimulated IL-4 levels in patients with atopic dermatitis (34.5 U/ml) than in healthy sub-

jects (1.2 U/ml). Their 19 patients with atopic dermatitis actually fall into two groups. Eight subjects' results were not different from those of control subjects, whereas 11 subjects had elevated mitogen-stimulated IL-4 production. Our subjects' results were consistent with those of the former group. These results point to the marked heterogeneity in the patterns of immune responsiveness in patients with atopic dermatitis. Furthermore, the correlation between IL-4 and IL-5 production in our patients with atopic dermatitis provides an internal control, confirming the validity of our cytokine measurements. Such a correlation was previously demonstrated by Parronchi et al.⁵⁶

It is noteworthy that our patients with atopic dermatitis had significantly higher in vitro IFN- γ levels than our healthy control subjects. Three previous studies reported a significantly lower in vitro IFN- γ production in patients with atopic dermatitis when compared with control subjects.^{21,55,57} Different mitogenic stimulants were used in each of these studies to induce in vitro IFN- γ production. We used ionomycin and phorbol myristate acetate, which act independently of antigen-presenting cells, as the mitogens. Del Prete et al.⁵⁵ used phytohemagglutinin, which is accessory cell-dependent. Jujo et al.²¹ performed two sets of cultures with different classes of mitogens. The former, a concanavalin A-stimulated culture (also accessory cell-dependent), showed lower IFN- γ values for a majority of patients with atopic dermatitis (mean = 259 ± 225 U/ml) compared with healthy control subjects (mean = 659 ± 139 U/ml). However, the latter, a phorbol 23, 13-dibutyrate and ionophore-stimulated culture, showed values for only three subjects to be more than 2 standard deviations (our calculations from their figure) below the mean of their normal values. Chan et al. also performed two sets of cultures with both accessory cell-dependent (anti-CD3) and accessory cell-independent (phorbol myristate acetate plus ionophore) stimulants. They report lower IFN- γ production by PBMCs from patients with atopic dermatitis in both sets of experiments. However, the variability in the latter set of cultures suggests a major overlap between the two groups. When antigen-presenting cells were removed, T cells from patients with atopic dermatitis clearly produced more IFN- γ than did those of control subjects.⁵⁷ The phorbol ester-stimulated IFN- γ production in the latter two reports is not inconsistent with our own results and suggests a role for an accessory cell factor in explaining the previously observed differences be-

tween healthy subjects and patients with atopic dermatitis. Prostaglandin E is likely to be one such mediator.^{57,58}

Our data raise questions regarding the frequency of T_{H1} versus T_{H2} lymphokine profile T cells in the circulation of patients with atopic dermatitis. The current paradigm is that atopic dermatitis is dominated by T_{H2} CD4+ cells, on the basis of the clinical findings of elevated circulating IgE and eosinophil concentrations¹ and the well documented generation of T_{H2} clones from lesional skin.^{24,25} However, the cellular constitution of our patients' circulating blood may not reflect their lesional cutaneous cell distribution or the fact that their lesional skin also contained T_{H1} cells, as found in patients with nonacute atopic dermatitis.^{49,50}

The processes driving IgE production in atopic dermatitis are multifactorial. IgE production can vary with the intensity of allergen exposure. Therefore the level of spontaneous in vitro IgE production may have too much temporal variation to allow observation of a consistent effect of IFN- γ treatment. This study demonstrated a trend toward a correlation between serum IgE and in vitro IgE levels in patients with atopic dermatitis, as demonstrated in other diseases.³¹ This study also demonstrates that diminished IFN- γ or excessive IL-4 production is not sufficient to explain the increase in serum IgE levels in patients with atopic dermatitis. Alterations in other cytokines or prostaglandin E synthesis,^{57,58} which modulate IgE production, rather than changes in IL-4 or IFN- γ production, may account for the dysregulation of IgE synthesis in atopic dermatitis. More knowledge of the interaction of these other cytokines in conjunction with IL-4 and IFN- γ in regulating the IgE response in atopic dermatitis is necessary to better understand the pathogenesis of this disease.

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REFERENCES

1. Cooper KD. Atopic dermatitis: recent trends in pathogenesis and therapy. *J Invest Dermatol* 1994;102:128-37.
2. Hoffman DR, Yamamoto FY, Geller B, Haddad Z. Specific IgE antibodies in atopic eczema. *J ALLERGY CLIN IMMUNOL* 1975;55:256-67.
3. Welbourn E, Champion RH, Parish WE. Hypersensitivity to bacteria in eczema. I. Bacterial culture, skin tests and immunofluorescent detection of immunoglobulins and bacterial antigens. *Br J Dermatol* 1976;94:619-32.
4. Walsh GA, Richards KL, Douglas SD, Blumenthal MN. Immunoglobulin E anti-*Staphylococcus aureus* antibodies in atopic patients. *J Clin Microbiol* 1981;13:1046-8.
5. Abramson JS, Dahl MV, Walsh G, Blumenthal MN, Doug-

- las SD, Quie PG. Antistaphylococcal IgE in patients with atopic dermatitis. *J Am Acad Dermatol* 1982;7:105-10.
6. Sampson HA. Role of immediate food hypersensitivity in the pathogenesis of atopic dermatitis. *J ALLERGY CLIN IMMUNOL* 1983;71:473-80.
7. Sampson HA, McCaskill CM. Food hypersensitivity and atopic dermatitis. Evaluation of 113 patients. *J Pediatr* 1985;107:669-75.
8. Mitchell EB, Crow J, Williams G, Platts-Mills TA. Increase in skin mast cells following chronic house dust mite exposure. *Br J Dermatol* 1986;114:65-73.
9. Mudde GC, Van Reijssen FC, Boland GJ, DeGast GC, Bruijnzeel PLB, Bruijnzeel-Kooman CAFM. Allergen presentation by epidermal Langerhans' cells from patients with atopic dermatitis is mediated by IgE. *Immunology* 1990;69:335-41.
10. Kieffer M, Bergbrant IM, Faergemann J, et al. Immune reactions to *Pityrosporum ovale* in adult patients with atopic and seborrheic dermatitis. *J Am Acad Dermatol* 1990;22:739-42.
11. Seikdenari S, Manzini BM, Danese P, Giannetti A. Positive patch tests to whole mite culture and purified mite extracts in patients with atopic dermatitis, asthma and rhinitis. *Ann Allergy* 1992;69:201-6.
12. Sampson HA, Jolie PL. Increased plasma histamine concentrations after food challenges in children with atopic dermatitis. *N Engl J Med* 1984;311:372-6.
13. Saryan JA, Leung DYM, Geha RS. Induction of human IgE synthesis by a factor derived from T cells of patients with hyper-IgE states. *J Immunol* 1983;130:242-7.
14. Romagnani S, Maggi E, Del Prete FG, Ricci M. IgE synthesis in vitro induced by T cell factors from patients with elevated serum IgE levels. *Clin Exp Immunol* 1983;52:85-8.
15. Nutman TB, Volkman DJ, Hussain R, Fauci AS, Ottesen EA. Filarial parasite-specific T cell lines: induction of IgE synthesis. *J Immunol* 1985;134:1178-84.
16. Sherr EH, Stein LD, Dosch HM, Saxon A. IgE-enhancing activity directly and selectively affects activated B cells: evidence for a human IgE differentiation factor. *J Immunol* 1987;138:3836-43.
17. Hassner A, Saxon A. Inhibition of ongoing myeloma IgE synthesis *in vitro* by activated human T cells. *J Immunol* 1983;130:1567-72.
18. Leung DYM, Brozek C, Frankel R, Geha RS. IgE-specific suppressor factors in normal human serum. *Clin Immunol Immunopathol* 1984;32:339-50.
19. Del Prete G, Maggi E, Parronchi P, et al. IL-4 is an essential factor for the IgE synthesis induced in vitro by human T cell clones and their supernatants. *J Immunol* 1988;140:4193-8.
20. Maggi E, Del Prete GF, Tiri A, et al. Role of interleukin-4 in the induction of human IgE synthesis and its suppression by interferon-gamma. *Ricerca Clin Lab* 1987;17:363-7.
21. Jujo K, Renz H, Abe J, Gelfand E, Leung D. Decreased interferon gamma and increased interleukin-4 production in atopic dermatitis promotes IgE synthesis. *J ALLERGY CLIN IMMUNOL* 1992;90:323-31.
22. Reinhold U, Wehrmann LO, Kukul S, Kreysel HW. Evidence that defective interferon-gamma production in atopic dermatitis patients is due to intrinsic abnormalities. *Clin Exp Immunol* 1990;79:374-9.
23. Furue M, Ohtsuki M, Ogata F, Ishibashi Y. Responsiveness to interleukin 4 and interleukin 2 of peripheral blood mononuclear cells in atopic dermatitis. *J Invest Dermatol* 1991;96:468-72.
24. Kapsenberg ML. High frequency of IL-4 producing CD4+ allergen-specific T lymphocytes in atopic dermatitis lesional skin. *J Invest Dermatol* 1991;97:389-94.
25. Mudde GC, Van Reijssen FC, Bruijnzeel-Kooman CAFM. IgE positive Langerhans cells and TH2 allergen-specific T cells in atopic dermatitis. *J Invest Dermatol* 1992;99:103S.
26. Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J Immunol* 1986;136:2348-57.
27. Cherwinski HM, Schumacher JH, Brown KD, Mosmann TR. Two types of mouse helper T cell clone. III. Further differences in lymphokine synthesis between Th1 and Th2 clones revealed by RNA hybridization, functionally monospecific bioassays and monoclonal antibodies. *J Exp Med* 1987;166:1229-44.
28. Boguniewicz M, Jaffe HS, Izu A, et al. Recombinant gamma interferon in treatment of patients with atopic dermatitis and elevated IgE levels. *Am J Med* 1990;88:365-70.
29. Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. *Acta Derm Venereol (Stockh)* 1980;92(suppl):44-7.
30. Hanifin JM, Schneider LC, Leung DYM, et al. Recombinant interferon-gamma therapy for atopic dermatitis. *J Am Acad Dermatol* 1993;28:189-97.
31. King CL, Poindexter RW, Ragunathan J, Fleisher TA, Ottesen EA, Nutman TB. Frequency analysis of IgE-secreting B lymphocytes in persons with normal or elevated serum IgE levels. *J Immunol* 1991;146:1478-83.
32. Limaye AP, Abrams JS, Silver JE, Ottesen EA, Nutman TB. Regulation of parasite-induced eosinophilia: selectively increased interleukin 5 production in helminth-infected patients. *J Exp Med* 1990;172:399-409.
33. Juhlin L, Johansson GO, Bennich H. Immunoglobulin E in dermatoses: levels in atopic dermatitis and urticaria. *Arch Dermatol* 1969;100:12-6.
34. Vercelli D. Regulation of IgE synthesis in man. *J Clin Immunol* 1989;9:75-83.
35. Saxon A, Ke Z, Bahati L, Stevens RH. Soluble CD23 containing B cell supernatants induce IgE from peripheral blood B-lymphocytes and costimulate with interleukin-4 in induction of IgE. *J ALLERGY CLIN IMMUNOL* 1990;86:333-44.
36. Kimata H, Yoshida A, Ishioka C, Lindley I, Mikawa H. Interleukin 8 (IL-8) selectively inhibits immunoglobulin E production induced by IL-4 in human B cells. *J Exp Med* 1992;176:1227-31.
37. Punnonen J, de Waal Malefyt R, van Vlasselaer P, Gauchet J-F, de Vries JE. IL-10 and viral IL-10 prevent IL-4 induced IgE synthesis by inhibiting the accessory cell function of monocytes. *J Immunol* 1993;151:1280-9.
38. Kiniwa M, Gately M, Gubler U, Chizzonite R, Fargeas C, Delespesse G. Recombinant interleukin-12 suppresses the synthesis of immunoglobulin E by interleukin-4 stimulated human lymphocytes. *J Clin Invest* 1992;90:262-6.
39. Wu CY, Brinkman V, Cox D, Heusser C, Delespesse G. Modulation of human IgE synthesis by transforming growth factor-beta. *Clin Immunol Immunopathol* 1992;62:277-84.
40. Pene J, Chretien I, Rousset F, Briere F, Bonnefoy JY, deVries JE. Modulation of IL-4-induced human IgE production *in vitro* by IFN-gamma and IL-5. The role of soluble CD23 (S-CD23). *J Cell Biochem* 1989;39:253-64.

41. Dugas B, Renauld JC, Pene J, et al. Interleukin-9 potentiates immunoglobulin (IgG, IgM, and IgE) production by normal human B lymphocytes. *Eur J Immunol* 1993;23:1687-92.
42. Punnonen J, Aversa G, Cocks BG, et al. Interleukin 13 induces interleukin 4-independent IgG₄ and IgE synthesis and CD23 expression by human B cells. *Proc Natl Acad Sci U S A* 1993;90:3730-4.
43. Vercelli D, Jabara HH, Lauener RP, Geha RS. IL-4 inhibits the synthesis of IFN gamma and induces the synthesis of IgE in human mixed lymphocyte cultures. *J Immunol* 1990;144:570-6.
44. Peleman R, Wu J, Fargeas C, Delespesse G. Recombinant interleukin 4 suppresses the production of interferon gamma by human mononuclear cells. *J Exp Med* 1989;170:1751-6.
45. Mosmann T, Coffman R. Th1 and Th2 cells: different patterns of lymphokine secretion lead to different functional properties of lymphokine secretion. *Annu Rev Immunol* 1989;7:145-73.
46. Gajewski T, Fitch F. Antiproliferative effect of IFN gamma in immune regulation. I. IFN gamma inhibits the proliferation of Th2 but not Th1 murine helper T lymphocyte clones. *J Immunol* 1988;140:4245-52.
47. Reinhold W, Kukul S, Kreysel H. Evidence that defective IFN-gamma production in atopic dermatitis patients is due to intrinsic abnormalities. *Clin Exp Immunol* 1990;79:374-9.
48. Ramb-Lindhauer C, Feldmann A, Rotte M, Neumann C. Characterization of grass pollen reactive T cell lines derived from lesional atopic skin. *Arch Dermatol Res* 1991;283:71-6.
49. Hamid Q, Boguniewicz M, Leung DYM. Differential in situ cytokine gene expression in acute versus chronic atopic dermatitis. *J Clin Invest* 1994;94:870-6.
50. Grewe M, Walther S, Gyufkoj K, Czech W, Schopf E, Krutmann J. The two-phase model for the pathogenesis of atopic dermatitis: further evidence. [Abstract]. *J Invest Dermatol* 1994;103:426.
51. Fiser PM, Buckley RH. Human IgE biosynthesis *in vitro*: studies with atopic and normal blood mononuclear cells and subpopulations. *J Immunol* 1979;123:1788-94.
52. Romagnani S, DelPrete GF, Maggi E, et al. In vitro production of IgE by human peripheral blood mononuclear cells. II. Cells involved in the spontaneous IgE production in atopic patients. *Clin Exp Immunol* 1980;42:579-88.
53. King CL, Gallin JI, Malech HL, Abramson SL, Nutman TB. Regulation of immunoglobulin production in hyperimmunoglobulin E-recurrent infection syndrome by interferon gamma. *Proc Nat Acad Sci* 1989;86:10085-9.
54. King CL, Ottesen EA, Nutman TB. Cytokine regulation of antigen-driven immunoglobulin production in filarial parasite infections in humans. *J Clin Invest* 1990;85:1810-5.
55. Del Prete G, Tiri A, Maggi E, et al. Defective in vitro production of gamma interferon and tumor necrosis factor alpha by circulating T cells from patients with the hyperimmunoglobulin E syndrome. *J Clin Invest* 1989;84:1830-5.
56. Parronchi P, Manetti R, Simonelli C, et al. Cytokine production by allergen (*Der p* 1)-specific CD4⁺ cell clones derived from a patient with severe atopic dermatitis. *Int J Clin Lab Res* 1991;21:186-9.
57. Chan SC, Kim J-W, Henderson WR Jr, Hanifin JM. Altered prostaglandin E₂ regulation of cytokine production in atopic dermatitis. *J Immunol* 1993;151:3345-52.
58. Byron KA, Liberatos S, Varigos GA, Wootton AM. Interferon-gamma production in atopic dermatitis: a role for prostaglandins? *Int Arch Allergy Immunol* 1992;99:50-5.

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