

Decreased interferon gamma and increased interleukin-4 production in atopic dermatitis promotes IgE synthesis

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The mechanism(s) responsible for increased IgE synthesis in atopic dermatitis (AD) are unknown, but they may be related to either decreased interferon gamma (IFN- γ) and/or increased interleukin (IL)-4 production. In this study we examined peripheral blood mononuclear cells (PBMCs) from 21 patients with AD, six patients with psoriasis, and 22 nonatopic healthy controls for IFN- γ and IL-4 production after stimulation with concanavalin A (Con A). The Con A-induced proliferative response of AD PBMCs was similar to the response of healthy controls ($p = 0.9$). After mitogen stimulation, however, AD culture supernatants contained significantly less IFN- γ ($p = 0.001$) but increased IL-4 ($p = 0.001$) compared with supernatants from nonatopic controls. In contrast, PBMCs from patients with psoriasis produced normal levels of IFN- γ and IL-4 in vitro. Since IL-4 is known to decrease IFN- γ synthesis, we examined the effect of neutralizing anti-IL-4 on IFN- γ production. Anti-IL-4 significantly increased IFN- γ production in patients with AD ($p = 0.008$) and nonatopic controls ($p = 0.02$) but did not normalize IFN- γ production by AD PBMCs. Supernatants from AD PBMCs, but not supernatants from nonatopic PBMCs, induced IgE synthesis in PBMCs from nonatopic donors ($p = 0.02$). When an anti-IFN- γ receptor antibody, which blocks cellular binding of IFN- γ , was added to supernatants from nonatopic controls their capacity to induce IgE synthesis was significantly greater ($p = 0.03$). These results demonstrate an imbalance of IL-4 and IFN- γ production, which may contribute to increased IgE synthesis in AD. (J ALLERGY CLIN IMMUNOL 1992;90:323-31.)

Key words: Atopic dermatitis, immunoglobulin E, skin, interleukin-4, interferon gamma

Atopic dermatitis (AD) is a chronic inflammatory skin disorder frequently associated with markedly elevated serum IgE levels.^{1,2} The basis for the increased IgE synthesis in AD is not completely understood, but is thought to be related to defects in T-cell regulation.³ Recently several important insights have

Abbreviations used:

AD:	Atopic dermatitis
Con A:	Concanavalin A
ELISA:	Enzyme-linked immunosorbent assay
IFN- γ :	Interferon gamma
IL:	Interleukin
PBMC:	Peripheral blood mononuclear cell
MCF:	Mean channel fluorescence
PBS:	Phosphate-buffered saline
PDB:	Phorbol-23, 13-dibutyrate
FCS:	Fetal calf serum

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been made into the regulation of IgE synthesis on human peripheral blood mononuclear cells (PBMCs). In particular, it has been demonstrated that interleukin-4 (IL-4) acts as an isotype switch factor that commits B cells to the IgE pathway.⁴ Additional signals such as cognate interaction with T cells as well as IL-5 and IL-6 are then required to obtain high levels of IgE synthesis.⁴⁻¹⁰

In contrast, interferon gamma (IFN- γ) inhibits *in vivo* and *in vitro* IgE synthesis in experimental animals as well as IL-4-induced IgE synthesis *in vitro* by PBMCs.^{4, 6-10} The cellular target by which IFN- γ inhibits IgE synthesis is currently unknown and appears to depend on the model system being studied. For example, Gauchat et al.⁴ reported that inhibition of functional C ϵ mRNA transcripts by IFN- γ in IL-4-induced peripheral blood lymphocytes required the presence of T cells. In contrast, IL-4-induced IgE synthesis by purified human B cells infected with Epstein-Barr virus can be suppressed by IFN- γ .⁵

The reciprocal effects by which IFN- γ and IL-4 in humans have generally been demonstrated *in vitro* are by use of recombinant cytokines on isolated PBMCs from healthy donors. It is therefore important to examine the potential *in vivo* relationship of these cytokines in atopic diseases. In the current study we examined whether low IFN- γ and/or elevated IL-4 production might be the basis for increased IgE synthesis in AD.

METHODS

Patients

Twenty-one patients with moderate to severe AD (median extent of skin involvement, 42%; range, 15% to 100%) and elevated serum IgE (serum IgE; median, 9847; range, 880 to 40682 U/ml) were studied. Their diagnosis was based on the criteria of Hanifin and Rajka.¹¹ All of the patients were highly atopic and had a personal history of either allergic rhinitis or asthma or both. None of the patients had other skin conditions nor had any received systemic steroids previously. Their topical medications had consisted of low and medium potency topical steroids. All patients were off antihistamines and topical steroids for 5 days before their blood donation. Informed consent was obtained from all subjects or from their parents before these studies were performed. Two control groups were studied; first, 22 healthy, nonatopic donors (serum IgE; median, 81; range, <5 to 198 U/ml); second, six patients with moderate to severe psoriasis (median extent of skin involvement, 45; range, 20% to 80% who were nonatopic (median serum IgE, 65; range, <5 to 170 U/ml). Topical steroids were not used for 5 days before blood donation by these patients.

Reagents

Recombinant IFN- γ (sp act: 2.02×10^7 U/mg) and mouse monoclonal antibodies (MAb) directed to different epitopes of IFN- γ (MAb20B8 and MG3099) were kindly provided by Dr. Baron Reed (Genentech, S. San Francisco). MAb GIR208, which recognizes the human IFN- γ receptor and blocks the binding of IFN- γ to its receptor, was obtained from Dr. Robert Schreiber (St. Louis). Recombinant human IL-4 was kindly provided by Dr. Paul Trotta (Schering Research Institute, Bloomfield, N.J.). A polyclonal rabbit antibody to IL-4 was obtained from Genzyme (Boston, Mass.). The concentration of anti-IL-4 used (1:750) represented the

amount of anti-IL-4 needed to neutralize 200 U/ml IL-4 in the functional Ramos B-cell assay (see herein).

Cell cultures for IgE synthesis

PBMCs from nonatopic donors were isolated from heparinized venous blood by density gradient centrifugation on Ficoll-Hypaque (Pharmacia Fine Chemicals, Piscataway, N.J.), washed three times in Hanks' balanced salt solution (Gibco; Grand Island, N.Y.), and resuspended at 1.0×10^6 cells/ml in HB 101 Liquid Basal (Irvine Scientific, Santa Ana, Calif.) supplemented with 10% heat inactivated fetal calf serum (FCS) (Hyclone Sterile Systems, Inc., Logan, Utah) supplemented with 2 mmol/L L-glutamine, 1.5 mmol/L sodium pyruvate, 15 mmol/L hepes, 50 μ g/ml streptomycin, and 100 U/ml penicillin. Cells were cultured at 37° C in a 5% CO₂ humidified atmosphere. Control cultures for the evaluation of preformed IgE were set up in the presence of cycloheximide (100 μ g/ml). Cytokines and antibodies were added as indicated for each experiment in the "Results" section. After 14 days, culture supernatants were harvested and assessed by ELISA for their IgE content as previously described.¹² Net IgE synthesis was evaluated by subtracting IgE concentrations found in cycloheximide-treated cultures from IgE values found in untreated cultures. Values for preformed IgE from these nonatopic donors, obtained with cycloheximide-inhibition for 14 days, were comparable to those obtained with the acid elution technique described by Turner et al.¹³

Preparation of PBMC supernatants for cytokine assays

PBMCs from patients with AD and healthy controls were suspended at 1×10^6 cells/ml in RPMI 1640-10% heat inactivated FCS supplemented with 2 mmol/L L-glutamine, 50 μ g/ml streptomycin, and 100 U/ml penicillin. Cells were stimulated with 10 μ g/ml concanavalin A (Con A, Boehringer Mannheim, Indianapolis, Ind.) or 10 nmol/L phorbol-23, 13-dibutyrate (PDB, Calbiochem, La Jolla, Calif.), and 0.5 mmol/L calcium ionophore, A23187 (Calbiochem). After 48 hours, the supernatants were harvested and stored at -80° C. These time intervals were associated with peak secretion of IFN- γ and IL-4 into culture supernatants.

Assay for IFN- γ production

IFN- γ concentrations were determined by use of an ELISA with a lower limit of sensitivity of 15 U/ml. Ninety-six well microtiter plates were coated with a purified monoclonal antihuman IFN- γ (MAb20B8; Genentech) diluted in 0.1 mol/L NaHCO₃ at a concentration of 5 μ g/ml. After that they were incubated at 37° C for 1.5 hours. Except for the substrate reaction, all subsequent steps were performed at 37° C, and phosphate-buffered saline (PBS) plus 0.05% Tween 20 was used for washing. After 1.5 hours incubation, the wells were washed and blocked with 2% bovine serum albumin (BSA) PBS for 1 hour. After washing, serially diluted supernatants were incubated in duplicate for 2 hours with parallel human IFN- γ standard controls. The plates

were subsequently washed and incubated for 1.5 hours with a horseradish peroxidase-conjugated monoclonal antihuman IFN- γ (MAbMG3099) diluted in PBS plus 0.05% Tween 20 at a concentration of 3 μ g/ml. After washing, the plates were developed with 0.2% mixture of peroxidase and 1.25 mg/ml of O-Dianisidine (DMB, Sigma Chemical Co., St. Louis, Mo.). After 30 to 45 minutes, 30 mm³ of 9 mol/L-H₂SO₄ was added to each well to stop the reaction. Optical density was read at 540 nm. The results obtained on all the culture supernatants from patients with AD and controls with use of this ELISA were confirmed by a commercially available IFN- γ RIA kit (Centocor, Malvern, Pa.).

Assay for IL-4 production

IL-4 could not be reliably measured by commercially available ELISA kits. We therefore assessed IL-4 content by the capacity of supernatants to induce CD23 on the CD23 negative Ramos B cell line based on a biologic assay by Custer and Lotze.¹⁴ Ramos cells were subcultured with RPMI 1640 in complete culture medium. For experiments, only viable cells obtained from Ficoll-Hypaque gradient centrifugation were used. Cells were cultured in 96-well tissue culture plates (Falcon Laboratories, Oxnord, Calif.) in a concentration as 1×10^5 cells per well and stimulated with 5 μ g/ml of anti-IgM (Tago, Burlingame, Calif.). Either recombinant human IL-4 in a concentration range between 1 and 400 U/ml or dilutions of tissue culture supernatants were added for 48 hours. Cells were washed twice and reincubated with human immunoglobulins at 5 mg/ml to prevent nonspecific binding by Fc receptor. Subsequently, the cells were incubated with a fluoresceinated monoclonal (IgG1, no. BU38) anti-CD23 antibody (Binding Site, San Diego, Calif.) for 30 minutes in staining buffer (PBS supplemented with 2% heat inactivated FCS and 0.1% sodium azide). After washing three times, CD23 expression was analyzed by flow cytometry on an EPICS Profile Cytofluorograph (Coulter, Hialeah, Fla.). Mean channel fluorescence (MCF) for CD23 expression was determined for each sample and standard. A standard curve was then established for each assay by plotting of CD23 MCF-value against corresponding IL-4 concentrations of standard samples on a linear scale. With use of this standard curve, MCF values of samples analyzed was transformed into IL-4 concentrations. The lower limit of sensitivity of this assay was 1 U/ml. The specificity of this assay was demonstrated by the loss of CD23 induction on Ramos cells when samples were preincubated with a polyclonal anti-IL-4 antibody (Genzyme). To prevent any potential effects of IFN- γ , all samples were tested in the presence of GIR208, an MAb that blocks the binding of IFN- γ to its receptor.

Proliferation assay

2×10^5 PBMCs per 0.2 ml culture medium were added in triplicate to 96-well flat bottom microtiter plates. After 3 days of mitogen stimulation, thymidine incorporation was assessed by pulsing with 0.5 μ Ci methyl-[³H]-thymidine (Amersham, Arlington Heights, Ill.) during the last 16 hours of culture.

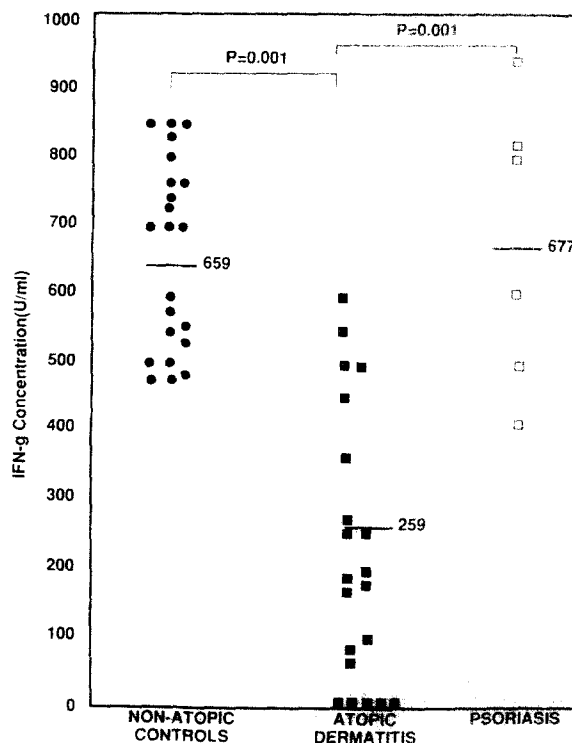


FIG. 1. PBMCs from patients with AD produce low levels of IFN- γ . PBMCs from patients with AD, patients with psoriasis, and healthy controls were stimulated with Con A for 2 days. Culture supernatants were then assayed for IFN- γ content. The lower limit of this assay was 15 U/ml. AD supernatants contained significantly less IFN- γ than control supernatant ($p = 0.001$).

Statistical analysis

Data were analyzed by two-tailed Student's t test for unpaired samples.

RESULTS

Decreased IFN- γ synthesis by PBMCs from patients with AD

To investigate IFN- γ synthesis in patients with AD, we assessed the effect of mitogens on IFN- γ production in vitro by PBMCs from patients with AD. As shown in Fig. 1, the mean Con A-induced IFN- γ production by PBMCs from 21 patients with AD (mean $\pm$ SD = 259 $\pm$ 225 U/ml) was significantly lower compared with the group of 22 controls (mean $\pm$ SD = 659 $\pm$ 139 U/ml; $p = 0.001$). Indeed, the in vitro IFN- γ production by PBMCs from 16 patients with AD were two standard deviations below the normal mean. IFN- γ production demonstrated a significant inverse correlation with serum IgE ($r = 0.55$, $p = 0.02$). Furthermore, when PBMCs were stimulated with PDB and the calcium ionophore, A23187, PBMCs from patients with AD made significantly less IFN- γ production than did healthy con-

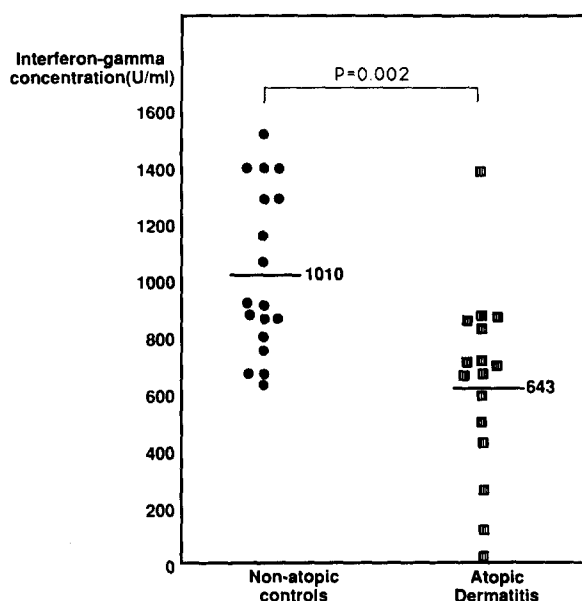


FIG. 2. After stimulation with PDB/ionophore, patients with AD synthesized significantly lower ($p = 0.002$) levels of IFN- γ than controls.

trols ($p = 0.002$) (Fig. 2). Since PDB and A23187 bypass cell surface signaling by directly stimulating protein kinase C, these data exclude a signal transduction defect to Con A in these patients as a basis for low IFN- γ production.

Basis of low IFN- γ production in AD

To determine whether the decreased IFN- γ production by PBMCs from patients with AD were simply due to their diffuse skin inflammation, we also examined IFN- γ production by PBMCs from six nonatopic patients with moderate to severe psoriasis. Our results indicate that after Con A stimulation PBMCs from patients with psoriasis produce similar amounts of IFN- γ in vitro as compared with healthy controls ($p = 0.9$). More importantly, PBMCs from patients with AD produce significantly less IFN- γ in vitro than do PBMCs from patients with psoriasis ($p = 0.001$).

The deficient IFN- γ production was not due to a defect in lymphocyte proliferation responses to Con A. As shown in Table I, Con A induced the same level of ^3H -thymidine incorporation in controls (mean $\pm$ SD, ^3H -thymidine incorporation = $86,570 \pm 7111$) and patients with AD (mean $\pm$ SD, ^3H -thymidine incorporation = $86,089 \pm 10241$; $p = 0.9$). Furthermore, PBMCs from controls and patients with AD showed similar levels of proliferation after stimulation with PDB and the calcium ionophore, A23187 ($p = 0.7$).

Recent studies have demonstrated that IL-4 inhibits

TABLE I. Con a and PDB/ionomycin induce the same level of ^3H -thymidine incorporation in controls and patients with AD

Stimulation	^3H -thymidine incorporation (cpm)		
	Controls	Atopic dermatitis	p Value
None	1542 ± 161	1407 ± 160	0.6
Con A	86570 ± 7111	86089 ± 10241	0.9
PDB + Ionomycin	97548 ± 6326	92076 ± 9917	0.7

IFN- γ production.^{15, 16} Therefore we asked whether the low IFN- γ levels observed in AD were caused by increased IL-4 production. In this regard, we assayed IL-4 production in supernatants of Con A activated cells from patients with AD versus healthy controls. As shown in Fig. 3, PBMCs from patients with AD (mean IL-4 = 34.5 U/ml) secreted significantly higher levels of IL-4 than nonatopic controls (mean, 1.2 U/ml ; $p = 0.001$) and patients with psoriasis (mean, 2.2 ; $p < 0.05$). This biologic assay for IL-4 was not influenced by the addition of exogenous IgE in amounts up to 50 ng/ml (Fig. 4). Thus any IgE in AD supernatants should not have influenced the IL-4 assay.

To further examine this issue, we assessed the effect of neutralizing polyclonal anti-IL-4 on IFN- γ production. As shown in Fig. 5, anti-IL-4 significantly enhanced IFN- γ production by both normal ($p = 0.02$) and AD PBMCs ($p = 0.008$). However, even in the presence of anti-IL-4, Con A-induced IFN- γ production by PBMCs from patients with AD was significantly lower than healthy controls ($p = 0.001$). Thus the low IFN- γ production in AD did not appear to be caused exclusively by the excessive production of IL-4. These observations are not surprising because sequential analysis of culture supernatants from PBMCs of healthy patients and patients with AD indicate that after Con A stimulation IFN- γ and IL-4 follow the same time course of production. Forty-eight hours was the peak time for release of both IFN- γ and IL-4 in these cultures (data not shown).

Decreased IFN- γ and increased IL-4 promote IgE synthesis in AD

We next asked whether the reduced IFN- γ and increased IL-4 production by AD PBMCs could account for elevated IgE levels in this disease. We therefore examined whether there was any difference in the

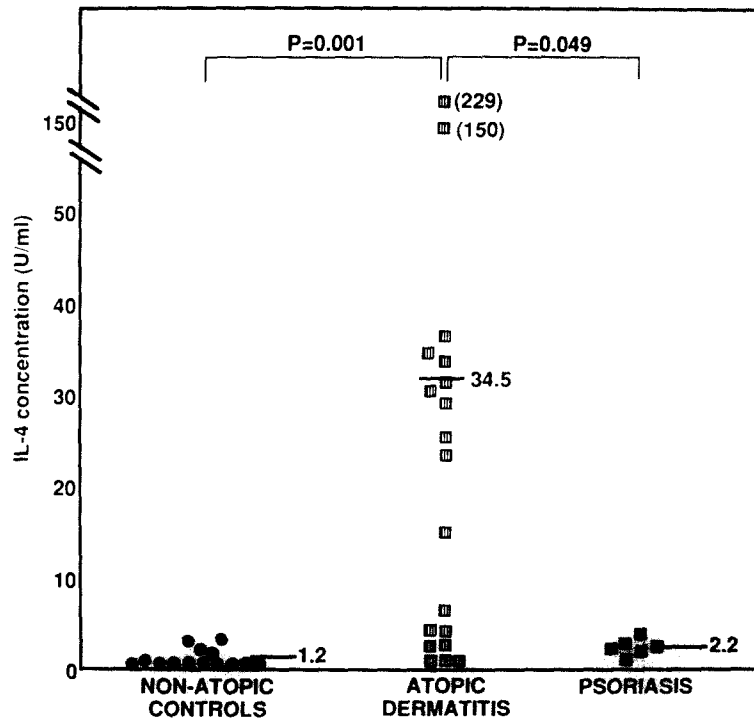


FIG. 3. PBMCs from patients with AD produce increased IL-4. PBMCs from patients with AD, patients with psoriasis, and healthy controls were stimulated with Con A for 2 days. Culture supernatants were then assayed for IL-4 content as described in the "Methods" section. The lower limit of this assay was 1 U/ml. AD supernatants contained more levels of IL-4 than supernatants from healthy patients ($p = 0.001$) and supernatants from patients with psoriasis ($p = 0.049$).

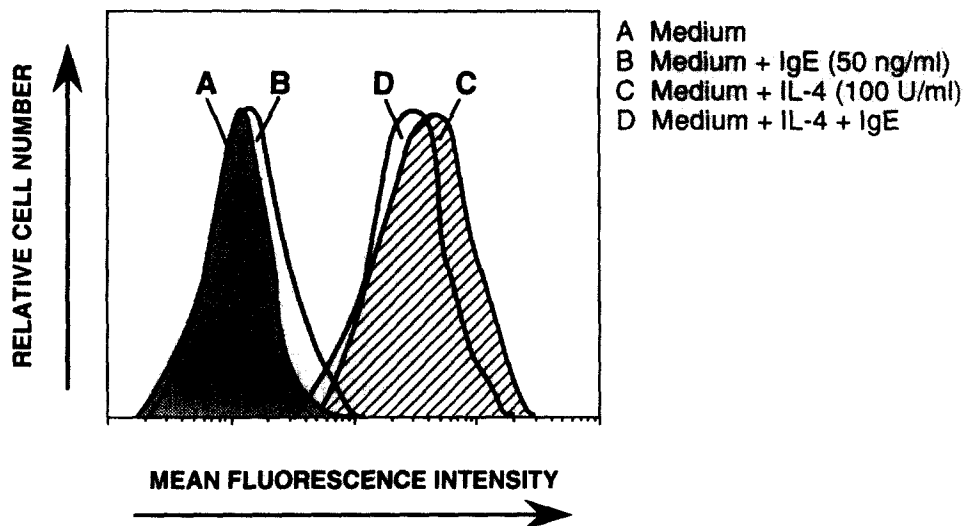


FIG. 4. Effect of exogenous IgE on IL-4-induced expression of CD23. In these experiments the Ramos B cell line was incubated in the presence of (A) medium alone, (B) medium with 50 ng/ml myeloma IgE(PS), (C) medium with IL-4(100U/ml), and (D) medium with IL-4 and myeloma IgE. After 48 hours of incubation at 37° C, the Ramos B cells were stained with fluoresceinated anti-CD23 and MCF for CD23 expression was determined on an EPICS profile cytofluorograph. The results indicate that in the presence and absence of exogenous IgE, IL-4 induces similar levels of CD23 expression.

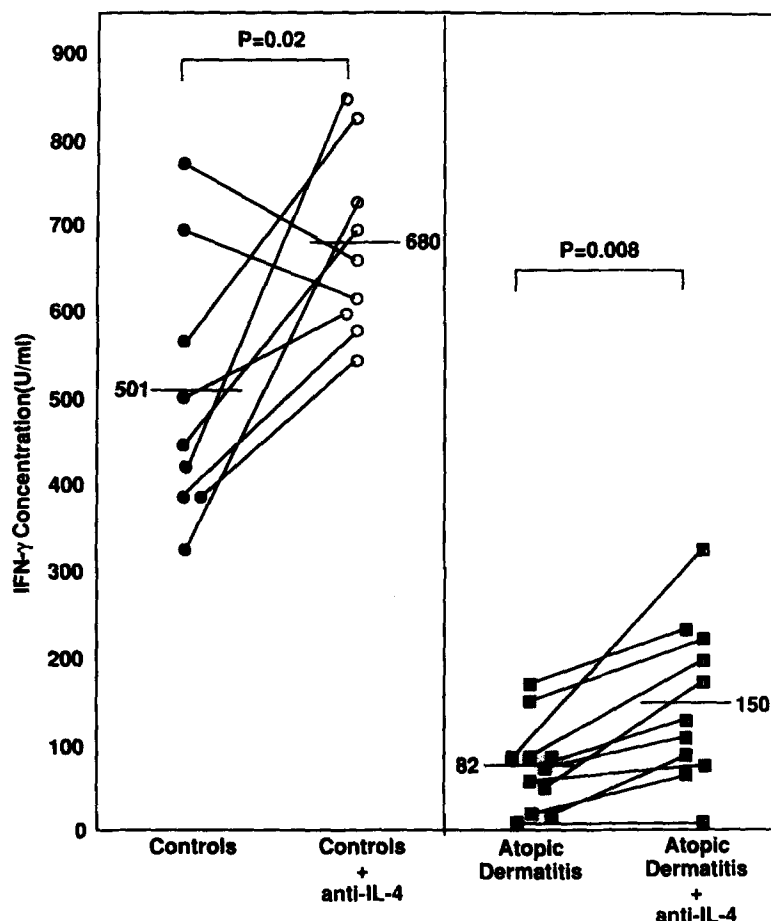


FIG. 5. Effect of anti-IL-4 on the production of IFN- γ . PBMCs from AD and healthy controls were stimulated with Con A for 2 days in the presence and absence of anti-IL-4. Culture supernatants were then assayed for IFN- γ content. Anti-IL-4 significantly enhanced IFN- γ production by PBMCs from controls ($p = 0.02$) and patients with AD ($p = 0.02$). However, even in the presence of anti-IL-4, Con A-induced IFN- γ production by PBMCs from patients with AD was significantly lower than healthy controls ($p = 0.001$).

capacity of supernatants from patients with AD versus nonatopic healthy controls to induce IgE synthesis by PBMCs from healthy nonatopic donors (Table II). Indeed, we found that supernatants from patients with AD that contained low IFN- γ and increased IL-4 content induced significantly higher levels of IgE than supernatants from healthy controls ($p = 0.02$). This IgE inducing activity by AD supernatants was decreased in the presence of anti-IL-4 (Table II).

Since IFN- γ has been demonstrated to inhibit IgE synthesis, we tested the hypothesis that the high levels of IFN- γ in healthy control supernatants prevented the induction of IgE in the last set of experiments. We therefore examined whether an antibody, GIR 208, which blocks the binding of IFN- γ to the human IFN- γ receptor allowed supernatants from controls to induce IgE. As shown in Table II, when GIR 208 was

added to these supernatants, they were able to induce significantly higher levels of IgE synthesis ($p = 0.03$). In contrast, AD supernatants induced IgE synthesis in the presence or absence of the anti-IFN- γ receptor. This was not surprising because AD supernatants contain low levels of IFN- γ .

DISCUSSION

Recent studies have demonstrated that the induction of IgE synthesis in human B cells requires a series of signals: first, the B cell must receive an early activation signal either via cognate interaction between the T cell receptor/CD3 complex on CD4⁺ T cells,¹⁷ and the MHC class II antigen plus peptide on B cells or a direct B-cell activator such as anti-CD40.^{18, 19} Second, the T-cell-derived lymphokine, IL-4, causes isotype switching of early B cells to IgE antibody

production.⁴⁻¹⁰ IL-4 dependent IgE synthesis is subsequently amplified by other lymphokines such as IL-5, a B-cell growth factor, and IL-6, a B cell differentiation factor.^{20, 21} In vitro and in vivo studies in rodents,⁷⁻⁹ and in vitro studies with human PBMCs have shown that IFN- γ can inhibit IgE synthesis induced by IL-4.^{4, 6, 10} Furthermore, it has been reported that the capacity of human and mouse T-cell clones to induce IgE synthesis is directly correlated with the ratio of secreted IL-4 to IFN- γ .^{6, 7} Previous studies examining long-term T-cell clones cultured from atopic human donors have, however, involved only a few donors.²²⁻²⁴ It is therefore not clear that such observations reflect the IL-4 and IFN- γ production of a general population of individuals.

In the present study we investigated 21 patients with AD for evidence of an imbalance of IFN- γ and IL-4 production. Our results indicate that after mitogen stimulation, PBMCs from patients with AD secreted decreased levels of IFN- γ and increased amounts of IL-4. These data confirm and extend the observations made by Reinhold et al.²⁵ and Rousset et al.²⁶ who also found that mitogen-stimulated PBMCs from patients with AD produced significantly less IFN- γ than PBMCs from nonatopic donors. In contrast, using limiting dilution cloning of three patients with AD, Delprete et al.²² concluded that patients with AD had normal numbers of IFN- γ and IL-4 producing T cells. In their report, however, they found that the number of IFN- γ producing cells from all three patients with AD were less than one SD below normal, and one of these three patients with AD had abnormally low number of IFN- γ producing cells. As already mentioned, since the clones were derived from only three donors, and the limiting dilution cloning required prolonged culturing in the presence of feeder cells, we believe that the data from their study are not conclusive.²²

Because our data on patients with AD could potentially be confounded by the secondary effects of chronic topical steroid usage or diffuse inflammatory skin disease, we also examined the capacity of PBMCs from nonatopic patients with psoriasis to produce IFN- γ and IL-4 in vitro. The patients with psoriasis were selected to have a similar extent of skin disease and topical steroid usage as our patients with AD. Our results indicate that compared with AD, PBMCs from patients with psoriasis produce significantly greater amounts of IFN- γ ($p = 0.001$) and significantly lower amounts of IL-4 ($p = 0.049$).

Recent studies have demonstrated that the exogenous addition of IL-4 to mitogen-stimulated PBMCs from healthy donors results in decreased IFN- γ mRNA expression and decreased IFN- γ secre-

TABLE II. Culture supernatants from healthy donors but not donors with AD require the presence of anti-IFN receptor (Rc) to enhance IgE synthesis by PBMCs from healthy donors

Donor of SUPS	+ Ig control	anti-IFN-Rc*
NL-1	0	2.7
NL-2	0	3.4
NL-3	0	6.3
NL-4	0	2.0
AD-1	7.0	6.6
AD-2	6.4	6.6
AD-3	21.5	24.0
AD-4	11.6	9.6

*A significant augmentation of IgE synthesis in PBMCs from non-atopic donors was observed after the addition of anti-IFN receptor to supernatants from healthy donors ($p = 0.03$) but not to supernatants from patients with AD ($p = 0.9$). Preformed IgE as determined by addition of cycloheximide to parallel cultures or acid elution of day 0 cell pellet (13) was less than 0.5 ng/ml.

tion.^{15, 16} Indeed, in our present study we observed that a neutralizing polyclonal anti-IL-4 significantly enhanced IFN- γ production by PBMCs from both healthy controls and those with AD. However, IFN- γ production by AD PBMCs remained significantly lower than normal. Our data suggest that the low IFN- γ production by PBMCs from patients with AD may be caused only in part by their increased IL-4 production.

We postulate that a dualistic mechanism for increased IL-4 and decreased IFN- γ may be operative in AD such that the excessive production of IL-4 by patients with AD does partly contribute to the observed decreased production of IFN- γ . However, we postulate that these patients also have a lower frequency of IFN- γ producing cells. The latter may be due to a defect in T-cell differentiation or the selective activation of IL-4 but not IFN- γ producing T cells. Indeed, a recent report by Wierenga et al.²³ observed that dust mite-specific T-cell clones derived from two patients with AD produced IL-4 but not IFN- γ , whereas dust mite-specific clones derived from a non-atopic individual produced IFN- γ but not IL-4. Although the latter studies certainly need to be extended to larger numbers of patients, they suggest that T cells from atopic individuals respond to the same allergen in a different manner than T cells from controls. The nature of the antigen or allergen may also play an important role in the cytokine production phenotype of T cells. In this regard, Parrouchi et al.²⁴ have reported that allergen-specific T cells derived from two atopic donors produced high levels of IL-4 but no IFN-

γ , whereas bacterial antigen-specific T-cell clones established from the same two donors produced predominantly IFN- γ but not IL-4.

To examine the potential relationship between elevated IgE synthesis in patients with AD and cytokine production, we examined the capacity of supernatants from the PBMCs of healthy donors and those with AD for their capacity to induce IgE synthesis in normal PBMC cultures. Our data indicate that supernatants from patients with AD induce significantly greater levels of IgE synthesis than supernatants from healthy controls. Indeed, supernatants from healthy donors were unable to induce the production of IgE unless an anti-IFN- γ receptor antibody, which has previously been shown to block cell binding of IFN- γ ,²⁷ was added to the cultures. These studies support the notion that IFN- γ plays a critical role in down-regulating IgE synthesis.

In conclusion, it would appear that decreased IFN- γ production and increased IL-4 production contributes to the increased IgE levels found in AD. Furthermore, activation of IFN- γ production is critical in maintaining low IgE levels in healthy donors. These data provide a rationale for use of IFN- γ as a therapeutic agent in the treatment of AD. Indeed, recent studies have demonstrated that the in vivo administration of recombinant IFN- γ to patients results in decreased spontaneous production of IgE by PBMCs as well as decreased clinical severity of skin disease caused by the AD.²⁸⁻³⁰

REFERENCES

- Wittig HJ, Belloit J, Fillipi ID, Royal G. Age-related serum immunoglobulin E levels in healthy subjects and in patients with allergic disease. *J ALLERGY CLIN IMMUNOL* 1980;66:305-13.
- Hoffman DR, Yamato FY, Geller B, Hadad Z. Specific IgE antibodies in atopic eczema. *J ALLERGY CLIN IMMUNOL* 1975;55:256-67.
- Leung, DYM, Geha, RS. Immunoregulatory abnormalities in atopic dermatitis. *Clin Rev Allergy* 1986;4:67-86.
- Gauchat J-F, Lebman DA, Coffman RL, Gascan H, deVries JE. Structure and expression of germline ϵ transcripts in human B cells induced by interleukin 4 to switch to IgE production. *J Exp Med* 1990;172:463-73.
- Thyphronitis G, Tsokos GC, June CH, Levine AD, Finkelman FD. IgE secretion by Epstein-Barr virus infected purified human B lymphocytes is stimulated by interleukin-4 and suppressed by interferon- γ . *Proc Natl Acad Sci U S A* 1989;86:5580-4.
- DelPrete 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.
- Coffman RL, Carty J. A T cell activity that enhances polyclonal IgE production and its inhibition by interferon- γ . *J Immunol* 1986;136:949-54.
- Snapper CM, Paul WE. Interferon- γ and B cell stimulatory factor-1 reciprocally regulate Ig isotype production. *Science* 1987;236:944-6.
- Finkleman FD, Katona IM, Mosmann TR, Coffman RL. IFN- γ regulates the isotypes of Ig secreted during in vivo humoral immune responses. *J Immunol* 1988;140:1022-6.
- Jabara HH, Ackerman SJ, Vercelli D, et al. Induction of interleukin-4-dependent IgE synthesis and interleukin-5-dependent eosinophil differentiation by supernatants of a human helper T cell clone. *J Clin Immunol* 1988;8:437-46.
- Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. *Acta Derm Venereol Suppl (Stockh)* 1980;92:44-7.
- Renz H, Mazer BD, Gelfand EW. Differential inhibition of T and B cell function in IL-4-dependent IgE production by cyclosporin A and methylprednisolone. *J Immunol* 1990;145:3641-6.
- Turner KJ, Holt PG, Holt BJ, Cameron KJ. *In vitro* synthesis of IgE by human peripheral blood leucocytes. III. Release of pre-formed antibody. *Clin Exp Immunol* 1983;51:387-94.
- Custer MC, Lotze MT. A biologic assay for IL-4. Rapid fluorescence for IL-4 detection in supernatants and serum. *J Immunol Meth* 1990;128:109-17.
- Peleman R, Wu J, Fargeas C, Delespessie G. Recombinant interleukin-4 suppresses the production of interferon γ by human mononuclear cells. *J Exp Med* 1989;170:1751-7.
- Vercelli D, Jabara HH, Lauener RP, Geha RS. IL-4 inhibits the synthesis of IFN- γ and induces the synthesis of IgE in human mixed lymphocyte cultures. *J Immunol* 1990;144:570-3.
- Vercelli D, Jabara HH, Arai K-I, Geha RS. Induction of human IgE synthesis requires interleukin 4 and T/B cell interactions involving the T cell receptor/CD 23 complex and MHC class II antigens. *J Exp Med* 1989;169:1295-9.
- Zhang K, Clark EA, Saxon A. CD40 stimulation provides an IFN- γ independent and IL-4-dependent differentiation signal directly to human B cells for IgE production. *J Immunol* 1991;146:1836-42.
- Jabara HH, Fu SM, Geha RS, Vercelli D. CD40 and IgE: synergism between anti-CD40 monoclonal antibody and interleukin 4 in the induction of IgE synthesis by highly purified human B cells. *J Exp Med* 1990;172:1861-4.
- Pene J, Rousset F, Briere F, et al. Interleukin-5 enhances interleukin-4-induced IgE production by normal human B cells. The role of soluble CD23 antigen. *Eur J Immunol* 1988;18:929-35.
- Vercelli D, Jabara HH, Arai K-I, Hokota T, Geha RS. Endogenous interleukin-6 plays an obligatory role in interleukin-4 dependent human (IgE). *Eur J Med* 1989;19:1419-25.
- DelPrete G, Tiri A, Maggi E, et al. Defective in vitro production of γ -interferon and tumor necrosis factor- α by circulating T cells from patients with hyper-immunoglobulin E syndrome. *J Clin Invest* 1989;84:1830-5.
- Wierenga EA, Snoek M, De Groot C, et al. Evidence for compartmentalization of functional subsets of CD4+ T lymphocytes in atopic patients. *J Immunol* 1990;144:4651-6.
- Parronchi P, Macchia D, Piccinni M-P, et al. Allergen- and bacterial antigen-specific T-cell clones established from atopic donors show a different profile of cytokine production. *Proc Natl Acad Sci U S A* 1991;88:4538-42.
- Reinhold U, Wehrmann W, Kukel S, Kreysel W. Evidence that defective interferon-gamma production in atopic dermatitis patients is due to intrinsic abnormalities. *Clin Exp Immunol* 1990;79:374-9.
- Rousset F, Robert J, Andary M, et al. Shifts in interleukin-4 and interferon- γ production by T cells of patients with elevated serum IgE levels and the modulatory effects of these lymphokines on spontaneous IgE synthesis. *J ALLERGY CLIN IMMUNOL* 1991;87:58-69.
- Sheehan KCF, Calderon J, Schreiber RD. Generation and char-

- acterization of monoclonal antibodies specific for the human IFN- γ receptor. *J Immunol* 1988;140:4231-6.
28. Boguniewicz M, Jaffe H, Izu A, et al. In vivo treatment of patients with atopic dermatitis and elevated IgE levels with recombinant gamma interferon. *Am J Med* 1990;88:376-9.
29. Parkin JM, Eales L-J, Galazka AR, Pinching AJ. Atopic manifestations in the acquired immune deficiency syndrome: response to recombinant interferon gamma. *BMJ* 1987;294:1185-6.
30. Schneider LC, Hanifin J, Cooper K, et al. Recombinant interferon gamma therapy reduces the clinical severity of atopic dermatitis. *J ALLERGY CLIN IMMUNOL* 1991;87:38A.

Prevalence of allergy to hymenoptera stings in different samples of the general population

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To get figures of prevalence of systemic reactions (SRs) to hymenoptera sting in adults between the ages of 18 to 65 years, we performed three different surveys using the same questionnaire from 1984 to 1988. The first one was a door-to-door survey in Southeast France, which included 8271 adults and used a questionnaire. The second one, which included 2067 adults, was performed in a health care center and comprised the same questionnaire and venom skin tests in subjects reporting a history of SRs. The third survey was a national poll performed through a home-based national computer network. The percentage of SRs ranged from 0.66% in the second survey including skin tests to 3.3% in the poll survey. The higher prevalence figure in this latter survey may be related to a false-positive history. There was no urban-rural difference in prevalence of SR. (J ALLERGY CLIN IMMUNOL 1992;90:331-4.)

Key words: Hymenoptera, allergy, anaphylaxis, systemic reaction, occurrence, epidemiology

Data on the prevalence of systemic reactions (SRs) to hymenoptera stings (HS) are scanty. Moreover, the subjects considered in many investigations have not been representative of the general population. Prevalence figures ranging from 0.15% to 3.9% have been reported.^{1,2} This wide variation may be related to a number of factors, that is, age, geographic location, definition of allergic reaction, and data gathering techniques (self-administered, standard questionnaire, or medical questionnaire). To obtain more accurate figures of prevalence of SR to HS in adults between the ages of 18 to 65 years, we performed three different surveys using the same questionnaire from 1984 to 1988.

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Abbreviations used

SR: Systemic reaction

HS: Hymenoptera sting

SUBJECTS IN THE STUDY AND METHODS

The first survey included 8271 adults, 18 to 65 years old, living in Southeast France: Marseille and three small towns nearby. These subjects were randomly selected from the general population by a three-stage cluster sampling in Marseille and simple random sampling in the surrounding towns. The questionnaire was administered door-to-door by medical students. It included four questions related to HS (Table I). In computing prevalence rates of SR, responses about local reactions were not considered. Only SRs listed on Table I were considered as such. Taking into account the mean refusal rate, which was 5%, and the mean absence rate of 10%, the overall response rate was approximately 85%.

The second survey was described by Charpin et al.³ In brief, it was performed in a public health care center in Marseille and included 2067 adults, 18 to 65 years old. The health care center provides free checkups on a 5-year basis