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Selective deficiency of interferon-gamma production in the hyper-IgE syndrome. Relationship to *in vitro* IgE synthesis

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SUMMARY

We measured the *in vitro* production of interferon-gamma (IFN-γ) in five cases of hyper-IgE syndrome (HIgE), induced by mitogens, calcium ionophores and phorbol ester. The biosynthesis of IFN-γ was severely reduced or undetectable in HIgE, while it was near normal in most atopic patients. The *in vitro* spontaneous production of IgE was increased overall in HIgE patients, although no correlation was found with serum IgE levels. Recombinant interleukin-4 (IL-4) induced a further increase in IgE synthesis, and its effect was totally antagonized by recombinant IFN-γ; the same pattern of response was also observed in atopic subjects with high production of IgE. IFN-α synergized with IL-4 on IgE synthesis, whereas recombinant IL-6 gave opposite changes in individual cases tested. We propose that IFN-γ deficiency may be responsible for some of the features of HIgE patients, including IgE levels and infections.

Keywords hyper-IgE interferon-gamma IgE synthesis interleukin-4

INTRODUCTION

The hyper-IgE (HIgE) syndrome, also termed Job's or Buckley's syndrome (Buckley, Wray & Belmaker, 1972; Donabedian & Gallin, 1983), is a rare form of primary immunodeficiency disease affecting monocytes, T and B lymphocytes (Donabedian & Gallin, 1983; Geha & Leung, 1989). The clinical manifestations of the disease include severe atopic eczema with onset in early childhood, recurrent pyogenic infections of the skin, characteristically due to *Staphylococcus aureus*, mucocutaneous candidiasis, and visceral abscesses. The distribution of eczematous lesions, however, differs from that observed in typical atopic dermatitis, since they usually affect also the head, face and extensor surfaces (Geha & Leung, 1989). The immunological abnormalities include extreme hyperimmunoglobulinaemia E, IgE antibodies to *Staph. aureus* and *Candida albicans* (Berger *et al.*, 1980; Matter *et al.*, 1986; Geha & Leung, 1989), presence of circulating immune complexes containing IgE combined to autoantibodies to IgE (Paganelli *et al.*, 1986; Quinti *et al.*, 1986), sometimes defective chemotaxis of polymorphs and monocytes, low T cell numbers, hypereosinophilia, and increased presence of FcER-II (CD23) on monocytes, B and perhaps T lymphocytes (Spiegelberg *et al.*, 1985). T lymphocyte proliferation in response to mitogens is normal or sometimes reduced in HIgE, and T cells produce factors capable of inducing significant IgE synthesis by normal B cells (Geha & Leung, 1989). These factors are found also in HIgE plasma, which contains IgE binding factors (BFs) (Leung *et al.*, 1986a).

IgE production is regulated by factors secreted by both B and T lymphocytes, including IgE BFs and cytokines such as interferon-gamma (IFN-γ) and interleukin-4 (IL-4) (Leung *et al.*, 1986b; Snapper & Paul, 1987). In particular, it is known both in mice and humans that IL-4 induces and potentiates *in vivo* and *in vitro* IgE biosynthesis, whereas IFN-γ counteracts IL-4-induced IgE production.

We found that five patients with HIgE syndrome had a severe deficiency of IFN-γ production *in vitro*, and increased spontaneous synthesis of IgE, which was further increased by addition of IL-4 but almost totally abolished by physiological amounts of IFN-γ.

PATIENTS AND METHODS

Patients

Five consecutive cases of HIgE syndrome, diagnosed according to the criteria established by Buckley & Becker (1978) and Geha & Leung (1989), were studied. There were four women and one man, with an age range of 4–26 years (median 7 years). All suffered from eczema, and two also from asthma. Recurrent deep-seated pyogenic infections from *Staphylococcus aureus* were present in all five; two also had herpes virus infections, and candidiasis was observed in four. Pneumonia developed in three cases. Eosinophilia (> 7% leucocytes, with absolute numbers

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ranging from 800 to 4000 per cubic mm) was present in all five. Preliminary investigations revealed impaired neutrophil and monocyte chemotaxis in three, and constantly high serum IgE levels in all five (patient no. 1 had 26 500 IU/ml, no. 2 had 37 000, no. 3 had 7500, no. 4 had 40 000, and no. 5 had 24 000). We did not look for specific IgE to *Staph. aureus*, but *Candida*-specific IgE were present in three out of four cases tested. Total T cell numbers were within the normal range, but an increased CD4/CD8 ratio was observed in some cases (patient no. 1 had a ratio of 4, no. 2 a ratio of 2·2, no. 3 a ratio of 1·9, no. 4 a ratio of 2·3, and no. 5 a ratio of 3·5). Two of the patients died, one of bowel cancer at the age of 12, and the other of a brain abscess at the age of 7.

Twenty atopic patients (five with eczema and 15 with asthma (one also with bronchopulmonary aspergillosis), and high levels of serum IgE) were studied as disease controls for *in vitro* IgE synthesis, IFN-γ production and effects of recombinant cytokines. The age range of these patients was 2–35 years, with a median of 14, and 13 of them were women. Fifteen healthy non-atopic young adults, nine of whom were men, aged between 17 and 51 years, chosen among the laboratory personnel, were studied as normal controls for the same parameters. Since IFN-γ production has not been reported to differ in young children and adults, and IgE production *in vitro* depends only on the atopic status and the type of stimulation, these controls were accepted as appropriate for this type of study.

Determination of IgE serum levels

These were assessed by standard paper radioimmunosorbent test (PRIST) purchased from Pharmacia Italy (Milan).

Mononuclear cell cultures

Lymphoprep-separated mononuclear cells from venous blood samples were resuspended in RPMI 1640 (GIBCO, Grand Island, NY) supplemented with penicillin–streptomycin (GIBCO), 100 U/ml, L-glutamine, 2 mM (GIBCO) and 10% v/v fetal bovine serum (Flow Laboratories) at 10^6 cells/ml. Duplicate cultures of 1 ml were set up for 8–14 days, with or without the following additions: cicloheximide (100 μg/ml), r-IL-4 (100 U/ml, a gift from S. Gillis, Immunex, Seattle, WA), IFN-γ (100 U/ml, a gift from Boehringer Ingelheim, Germany), r-IL-6 (100 U/ml, purchased from Genzyme, Boston, MA) and r-IFN-α 2a (RoferonA, Roche, Switzerland, 300 U/ml), and their combinations. The doses chosen were those reported by previous studies as giving optimal effects on IgE synthesis without toxic or other actions (Snapper & Paul, 1987; Pène *et al.*, 1988). In our hands, no significant differences between 100 and 1000 U/ml of either IL-4 or IFN-γ were recorded in three separate preliminary experiments. Supernatants were then collected and stored in aliquots at −20°C for up to 3 weeks until analysis for IgE determinations.

Induction of IFN-γ in vitro

Triplicate cultures of 2×10^5 cells/well were plated in 96-well plastic plates (Falcon, Oxnard, CA), with or without the following inducers: phytohaemagglutinin-P (PHA-P) (Wellcome, Beckenham, UK, 1 μg/ml), Staphylococcal enterotoxin B (SEB, 0·2 μg/ml), phorbol-myristate acetate (PMA, Sigma Chemical Co., St Louis, MO, 10 ng/ml), calcium ionophores A23187 (Sigma, 10 and 0·1 μM) and ionomycin (Squibb, Princeton, NJ, 0·05 μM). After 24 and 48 h the supernatants were collected and tested for IFN-γ production by an immunoradiometric assay (IFN-γ IRMA, Centocor, Malvern, PA) and by a bioassay as described previously (Matricardi *et al.*, 1984). There was a significant agreement between the two titration methods, as reported earlier (Paganelli *et al.*, 1988). As further control, IFN-α was induced with Newcastle disease virus (NDV) as described by Matricardi *et al.* (1984) and titrated by bioassay. In all those tested, IFN-α production was normal, as reported in other diseases (Matricardi *et al.*, 1984).

IgE determination

We established a very sensitive ELISA for measurement of low levels of IgE secreted *in vitro*, using monoclonal antibodies (MoAbs) A2·05·001 and ANA-B26 supplied by Dr E. A. Klasen (Biodata, Rome, Italy). The assay was done in 96-well PVC plates, coated with the first MoAb at 10 μg/ml in carbonate–bicarbonate buffer, pH 9·6, at 37°C for 2 h in a humidified chamber. After three washes with 0·9% NaCl containing 0·2% human serum albumin, the culture supernatants and the standards of IgE were added for 18 h at room temperature. After three more washes, the second MoAb conjugated with alkaline phosphatase was added, for 2 h at 37°C, followed by three washes with the same solution, and a fourth one with 0·1 M Tris–HCl, pH 8·2. The alkaline phosphatase substrate (Sigma) at 1 mg/ml was then delivered to the wells and developed for 30–90 min at room temperature. The OD readings of samples, obtained by a Titertek reader at 405 nm, in duplicates, were interpolated with a standard curve generated with dilutions, in RPMI with 10% v/v fetal bovine serum, of standard myeloma IgE supplied by Biodata as well as the standards of the IgE-Phadebas PRIST (Pharmacia). Lower detection limit in this system was 175 pg/ml of IgE.

For most samples, a modified PRIST was also used, as described by Hemady *et al.* (1983). Briefly, paper discs were incubated with 200 μl of the supernatants overnight, then washed and reincubated with 1/4 dilution of the Phadebas-RAST (Pharmacia) ^{125}I-labelled anti-IgE (200 μl volume) for 18 h. Bound radioactivity was read against dilutions of the IgE standards supplied, down to 150–300 pg/ml of IgE, depending on the test run.

The two methods did not show significant discrepancies of calculated results in the range 0·6–10 ng/ml.

In all subsequent calculations, the amount of IgE measured in cultures supplemented with cycloheximide (an inhibitor of protein synthesis) were subtracted from other calculated data, to give the net *de novo* IgE synthesis, disregarding the amount preformed and released because bound to cells (Ricci *et al.*, 1985; Del Prete *et al.*, 1986, 1988; Vercelli & Geha, 1989). This value varied from below detection (i.e. <0·2 ng/ml) in all controls, to 3 ng/ml in a patient with HIgE, indicating the release of preformed cell-bound IgE.

Statistical analysis

Data analysis was performed by Wilcoxon's and rank-sum tests.

RESULTS

In vitro *production of IFN-γ*

Table 1 shows the results of IFN-γ production in normal subjects, patients with atopy, including cases with atopic dermatitis and allergic asthma, and five cases with hyper-IgE

Table 1. Amounts of IFN-γ released in cultures stimulated with different inducers

	Age (years)	*In vitro* production of IFN-γ (U/ml) induced by: PHA	SEB	A23187 + PMA	Ionomycin + PMA
HIgE patients					
No. 1	4	<3	10	<3	<3
No. 2	6	<3	<3	<3	<3
No. 3	7	3	<3	20	ND
No. 4	12	20	30	ND	ND
No. 5	26	<3	<3	3	<3
Atopic controls ($n=12$)	2–35	55* (3–400)	98* (3–500)	40* (30–300)	40 (3–150)
Normal controls ($n=15$)	17–51	300* (30–2000)	250*† (3–3000)	100* (3–300)	100 (3–300)

Median and range of results given for controls.
* $P<0.001$ *vs* HIgE.
† $P<0.01$ *vs* atopic controls.

Table 2. Net spontaneous *in vitro* production of IgE by mononuclear cells from HIgE patients, and atopic controls

		Net IgE *in vitro* synthesis (ng/ml)
HIgE patients		
No. 1		12·5
No. 2		11·1
No. 5		4·2
Controls		
Atopic dermatitis	($n=4$)	1·68* (<0·2–3·78)
Respiratory allergy	($n=15$)	0·33† (<0·2–1·7)

Median and range given for atopic dematitis and respiratory allergy.
* $P<0.001$ *vs* respiratory allergy.
† $P<0.01$ *vs* HIgE.

syndrome. Different inducers were tested in almost all instances, and the findings revealed that a severe and significant decrease of IFN-γ *in vitro* production is present constantly in patients with HIgE, and occasionally in some patients with high levels of serum IgE and either atopic dermatitis or allergic asthma. However, in the latter cases, only two showed a consistent defect with all inducers, and the mean levels in each group were higher than those observed in HIgE patients. In two HIgE patients and nine atopics tested, the synthesis of IFN-α induced by NDV was in the normal range (1000–3000 U/ml).

In vitro *synthesis of IgE*

In 15 atopic patients with respiratory allergies (asthma with or without rhinitis) the net spontaneous production of IgE by mononuclear cells was in the range <0·2–1·7 ng/ml (see Table 2). The serum levels of IgE in these patients ranged from 300 to 4000 IU/ml (normal values < 150 IU/ml).

In cases with atopic dermatitis, serum IgE levels were between 620 and 8500 IU/ml, much higher than in respiratory allergies, also taking into account the lower mean age. The average mean of *in vitro* production of IgE was also higher, although not directly related to serum IgE values (this was also found in asthmatic patients). Only one out of 10 non-atopic controls produced detectable IgE spontaneously *in vitro* (0·3 ng/ml), and his serum IgE were above normal (210 IU/ml).

Patients with the HIgE syndrome showed enhanced spontaneous production of IgE in cultures, again unrelated to serum IgE values.

The addition of recombinant cytokines produced similar effects in atopics and HIgE patients and a summary of the results is shown in Table 3.

The addition of IL-4 resulted mainly in increased IgE *in vitro* synthesis, but this effect was not seen in a patient with HIgE. On the other hand, IFN-γ addition gave a consistent inhibition of *in vitro* IgE production, and its simultaneous addition with IL-4 totally abolished the enhancing effect of the latter cytokine (Table 3). This effect seems to be more potent in cases with low or undetectable IFN-γ production, but it can be observed also in the others (i.e. atopic controls nos 2, 3, 5, 6 and 7).

The addition of IFN-α2a to IL-4-stimulated cultures did not antagonize the enhancement of IgE synthesis, but it seemed rather to potentiate the IL-4-induced increase of IgE in four out of five patients. Recombinant IL-6 added at the beginning of cultures gave inconsistent and opposite results on IgE synthesis (Table 3).

DISCUSSION

We have shown that patients with the HIgE syndrome have defective *in vitro* production of IFN-γ, stimulated with several inducing agents.

Table 3. Net IgE production *in vitro* in presence of different cytokines, compared to spontaneous synthesis

	Net variation of IgE synthesis (ng/ml) induced by recombinant cytokines					
	Spontaneous	IL-4	IFN-γ	IL-4 + IFN-γ	IL-4 + IFN-α	IL-6
HIgE patients						
1.	12·5	20·2	8·9	11·8	21·1	14·3
2.	11·1	10·9	5·2	4·2	13·6	10·7
5.	4·2	5·6	4·1	4·7	ND	ND
Atopic controls						
1. AD	1·2	4·1	0·8	2·0	4·8	5·1
2. AD	3·8	10·9	3·2	3·0	ND	ND
3. AD	0·2	0·6	<0·2	<0·2	<0·2	0·2
4. RA	1·1	1·4	0·8	0·3	3·1	0·7
5. RA	1·7	3·9	1·6	1·5	ND	ND
6. RA	0·4	0·7	0·2	0·4	ND	ND
7. RA	0·3	0·5	0·2	0·3	ND	ND

ND, not done; AD, atopic dermatitis; RA, respiratory allergy.
Columns 1–4: $P < 0.03$ HIgE *vs* atopic controls.

IFN-γ is produced by T lymphocytes, probably a subset which has been characterized in mice as TH1 (Mosmann *et al.*, 1986), but still not clearly identified in humans (Bottomly, 1988; Lewis *et al.*, 1988). IFN-γ is not only an anti-viral agent, but it also regulates the expression of some surface molecules, increasing class II MHC products and decreasing CD23 expression. IFN-γ is also involved in the regulation of immunoglobulin synthesis, and in particular it suppresses IL-4-induced IgE synthesis, both in mice and humans (Snapper & Paul, 1987; Pène *et al.*, 1988).

In HIgE patients, where inducing stimuli fail to generate IFN-γ *in vitro*, we observed that addition of exogeneous IFN-γ to cultures for IgE synthesis resulted in a decrease of IgE. The simultaneous addition of IL-4 and IFN-γ, however, showed that IFN-γ was able to abrogate almost completely the enhancement of IgE production due to IL-4.

The inducers of IFN-γ we used are known to promote T cell activation and IFN-γ gene transcription in different ways, some requiring interaction with cellular surface molecules or receptors such as PHA (Valentine *et al.*, 1985) and SEB (Kappler *et al.*, 1989), others, such as calcium ionophores and phorbol esters, bypassing this step (Truneh *et al.*, 1985; Chatile *et al.*, 1989). The type of inducer was designed to characterize the failure of response, which was probable since we first identified defects of IFN-γ in two cases of HIgE in a survey of primary immune deficiencies (Matricardi *et al.*, 1984). We may argue from our data that the defect is not due to cell membrane defects, nor to defective macrophage–T cell co-operation (Paganelli *et al.*, 1988), since both inducers acting via cell membrane receptors and stimuli bypassing this step gave consistently low or absent production of IFN-γ. Also defects of second messengers seem not to be in question, since ionomycin and PMA can directly substitute for them, activating intracellular calcium mobilization and protein kinase C function. Therefore in cases with HIgE syndrome the deficient production of IFN-γ represents a primary feature of the cellular defect, a finding already observed in immunodeficient patients (Paganelli *et al.*, 1988). The presence of very low but measurable levels of IFN-γ in two patients' cultures after *in vitro* activation of mononuclear cells argues against gene deletion or mutation.

Since both CD4- and CD8-positive T lymphocytes can produce IFN-γ (Kasahara *et al.*, 1983) a deficiency of CD8 and/or CD4 producer subsets may explain our findings. An increased CD4/CD8 ratio is observed in HIgE patients, and confirmed in our cases, but the question of their contribution to lymphokine production has been only recently addressed by Del Prete *et al.* (1989) who analysed the production of IL-4 and IFN-γ by T cell clones from HIgE patients. A highly significant reduction of the IFN-γ-producing clones was found in HIgE irrespective of the CD4 or CD8 phenotype, with normal percentage of clones producing IL-4. Therefore, a disproportion between IL-4- and IFN-γ-producing subsets (Mosmann *et al.*, 1986; Del Prete *et al.*, 1986; Lewis *et al.*, 1988; Vercelli & Geha, 1989) seems to be the reason for the overproduction of IgE in these patients.

In cultures for spontaneous IgE synthesis, patients with HIgE produced IgE well above those measured in atopic subjects. Modulation by addition of exogeneous cytokines resulted in enhancement of IgE production with IL-4 in all cases, but the extent of the increase was very variable. A net decrease of IgE was found when IFN-γ was added either alone or together with IL-4. The balance of the opposing effects of IFN-γ and IL-4 on IgE production (Snapper & Paul, 1987) was shifted towards the inhibition of net IgE synthesis.

Recombinant IFN-α has been reported to inhibit *in vitro* IgE synthesis (Pène *et al.*, 1988). In our system, however, the predominant effect of rIFN-α, added together with r-IL-4, was to enhance the *in vitro* synthesis of IgE. Our results are at variance with those reported by Pène *et al.* (1988), and the only apparent difference seems to be the use of IFN-α2a in our experiments, and IFN-α2b in their study, at the same dose expressed in activity units.

In few cases, the addition of r-IL-6, previously named IFN-β2 (Kishimoto, 1989), showed that *in vitro* IgE synthesis could be affected in opposite ways by this cytokine. Since IL-6 seems to be the terminal differentiation factor of all B cells (Kishimoto,

1989), its action was expected to univocally increase *de novo* IgE synthesis in all patients. No conclusive evidence can be derived from our preliminary observation. It should be noted that both IFN-α (Matricardi *et al.*, 1984, and this paper) and IL-6 (unpublished observation) are normally produced in HIgE patients.

Defective IFN-γ production represents a feature of HIgE patients which is not present in the majority of atopic subjects (Del Prete *et al.*, 1989). The absence of IFN-γ, which negatively regulates IgE synthesis, strongly suggests that it may be the cause of the hyperimmunoglobulinaemia E in these patients. Its role in common atopy and other conditions with high IgE production has not been established, and there is conflicting evidence on IFN-γ production.

In the present study, IFN-γ production, although not absent, was reduced also in atopic patients (Table 1). This observation, though not reaching statistical significance except for SEB-induced IFN-γ, is partly in agreement with those described by Reinhold *et al.* (1990) in a selected series of patients with severe atopic dermatitis who had very reduced, and even absent, *in vitro* synthesis of IFN-γ. The levels produced were correlated with serum IgE, and this seems to be at variance with our data on a more heterogeneous population of atopic patients.

Inability to produce IFN-γ may also explain susceptibility to infections in HIgE patients. The use of rIFN-γ as replacement therapy in HIgE patients may therefore be postulated on the basis of *in vitro* findings. Very recently, Boguniewicz *et al.* (1990) reported encouraging data on treatment with rIFN-γ of patients with atopic dermatitis and elevated IgE levels. Unfortunately, they did not assess *in vitro* or *in vivo* production of IFN-γ in their patients, so we do not know whether the degree of benefit was related to the initial level of IFN-γ.

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