Regulation of immunoglobulin production in hyper-IgE (Job's) syndrome

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Background: The hyper-IgE (HIE), or Job's, syndrome is a rare, complex disorder characterized by high levels of serum IgE in childhood and chronic dermatitis with recurrent, often severe sinopulmonary and skin infections. Although the etiology of HIE syndrome is unknown, there is evidence that patients with HIE have abnormalities in cellular immune responses, as well as in the production of polyclonal and antigen-specific antibodies. Furthermore, there appears to be a common (but still undefined) mechanism underlying the regulation of IgE and IgG4 in this condition.

Objective: We sought to assess the role of cytokines or cytokine receptor blockade in regulating IgE and IgG4 production in HIE.

Methods: PBMCs were isolated from patients with HIE (n = 9) and normal individuals (n = 8), and IgE and IgG4 production was assessed spontaneously, in the presence of recombinant IL-4, IL-13, IL-6, IL-8, IL-12, and IFN-γ, under conditions in which the IL-4R was blocked or when these cytokines were neutralized by specific monoclonal or polyclonal antibodies.

Results: In PBMCs from patients with HIE, a significant ($P < .01$) reduction in the spontaneously produced IgE (and IgG4) was induced by either IFN-γ or IL-12, although neither cytokine could totally abrogate the immunoglobulin production. Whereas spontaneous IgE (and IgG4) production was not affected by exogenous IL-4 and IL-13, neutralizing antibodies to IL-4 and IL-13 also significantly ($P < .01$) reduced the production of IgE and IgG4, a finding supported by the observation of increased expression of IgE germline transcripts in these patients. In contrast to the neutralization of IL-4 and IL-13 protein, anti-IL-4R antibodies or soluble IL-4R completely suppressed IgE and IgG4 production in HIE. Similarly, IL-8 or antibodies to IL-6 and TNF-α, cytokines known to affect IL-4–dependent IgE production, completely inhibited both IgE and IgG4 production.

Conclusion: These data show that overproduction of IgE and IgG4 can be regulated by a number of cytokines affecting the IL-4–dependent pathway of IgE/IgG4 production in HIE and suggest new targets for therapeutic intervention. (J Allergy Clin Immunol 1999;103:333-40.)

Key words: *Cytokines, hyper-IgE, IFN-γ, IgE, IgG4, IL-4, IL-12, IL-13, Job's syndrome*

The hyper-IgE (HIE), or Job's, syndrome is a rare, complex disorder characterized by high levels of serum IgE and chronic dermatitis with recurrent, often severe sinopulmonary and skin infections. It is generally associated with coarse facies, kyphoscoliosis, and bone fragility.[1,2] A defect in neutrophil chemotaxis has often been reported in this syndrome.[3-5] Although the etiology of HIE syndrome is unknown, there is evidence that patients with HIE have abnormalities in cell function that include impaired delayed hypersensitivity responses to recall antigens and impaired in vitro T cell–mediated responses.[6,7] Furthermore, these patients have a defect in the production of antibody to specific antigen and to polysaccharides.[8-10] Although the total serum IgG values usually are within the normal range,[5,9] there appear to be elevated IgG4 levels,[8-11] suggesting a common (but still undefined) mechanism underlying the regulation of IgE and IgG4 in this condition. Very recently, a mutation in the α-subunit of the IL-4 receptor has been described in 3 patients with HIE. This mutation has been suggested to cause a gain in IL-4R–mediated function,[12] but it also may be a normal polymorphic allelic variant.[13]

The regulation of IgE synthesis in human subjects has been extensively investigated. The current consensus suggests that IgE production is dependent on 2 signals, the first resulting from a cognate interaction between membrane-bound receptors and ligands expressed by activated helper T and B lymphocytes and the second being from the T cell–derived cytokines IL-4 and/or IL-13.[14,15] Furthermore, the IL-4– or IL-13–dependent IgE production can be potentiated by a number of other cytokines, including IL-5, IL-6, IL-9, TNF-α, erythropoietin, and possibly IL-3; it can be diminished by other cytokines, such as IFN-γ, IFN-α, IL-12, transforming growth factor (TGF)-β, and IL-8.[16]

In situations in which IgE is elevated, IgG4 also tends to be elevated, which is felt to be a reflection of the regulatory controls provided by IL-4 and IL-13. Both IL-4 and IL-13 are capable of inducing the switching from μ to either ε or γ4 in naive B cells.[17,18] A number of patho-

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TABLE I. The production of IgE/IgG4 in vitro by PBMCs from patients with HIE is not affected by recombinant human IL-4 and IL-13 but is augmented by IL-6*

	IgE, ng/mL (% change)						IgG4, ng/mL (% change)					
Patient no	Spontaneous†	CHX‡	IL-4	IL-13	IL-4 + IL-13	IL-6	Spontaneous†	CHX‡	IL-4	IL-13	IL-4 + IL-13	IL-6
2	5.7	0	7.4 (+30)	7.1 (+25)	7.0 (+23)	7.3 (+28)	ND	ND	ND	ND	ND	ND
4	8.4	0	8.4 (0)	7.9 (–6)	8.4 (0)	9.9 (+18)	8.8	0	9.8 (+11)	8.0 (–9)	8.5 (–3)	11.2 (+27)
5	29.3	0	26.8 (–9)	26.2 (–11)	29.4 (+1)	41.8 (+41)	6.0	0	6.0 (0)	5.8 (–3)	5.1 (–15)	12.0 (+100)
6	ND	ND	ND	ND	ND	ND	2.3	0	2.5 (+9)	2.9 (+26)	2.5 (+9)	9.5 (+313)
9	5.1	0	5.2 (+2)	5.1 (0)	5.2 (+2)	6.3 (+24)	2.9	0	3.4 (+17)	2.8(–3)	3.0 (+3)	3.9 (+34)

ND, Not done.
*Patient PBMCs (10^6/mL) were cultured for 10 days in the presence or absence of IL-4, IL-13, IL-4 + IL-13, or IL-6 (100 IU/mL).
†Spontaneous production.
‡Immunoglobulin production in the presence of cycloheximide (100 μg/mL).

Abbreviations used
HIE: Hyper-IgE (Job's) syndrome
PMA: Phorbol myristate acetate

logic situations, such as allergic states,[19,20] nephrotic syndrome,[21] and helminth infections,[22] are characterized by parallel, elevated levels of IgE and IgG4, 2 isotypes that are characteristically found at extremely low concentration in body fluids. A number of molecules, such as those produced by activated B cells[23] or the regulatory cytokine IL-12,[24] have been shown to downregulate IgG4 or IgE production differentially, an issue that has potential interest in the understanding of immunoglobulin dysregulation, particularly because IgE and IgG4 mediate quite different functions.[22,25,26]

With an eye toward therapeutic intervention, this study demonstrates that the elevated spontaneous production of IgE/IgG4 is largely dependent on endogenous IL-4, IL-13, IL-6, and TNF-α. It also demonstrates that IL-12 and IFN-γ, although effective at lowering IgE/IgG4 production, are not nearly as potent as IL-8, sIL-4R, or neutralizing antibodies to IL-6 and TNF-α.

METHODS

Patient populations

All 9 patients with HIE included in this series satisfied the clinical criteria for HIE by having elevated serum IgE, chronic dermatitis, and documented deep-seated infections. The clinical and biologic characteristics of most of the patients have been reported previously.[1,11] For studies of IL-4 production, 7 nonallergic normal subjects with levels of IgE less than 100 IU/mL were also studied.

Isolation of cell populations

PBMCs from heparinized blood were obtained as described[11] and cryopreserved in liquid nitrogen until used in the study.

Culture conditions for in vitro antibody production

PBMCs were rapidly thawed, washed, and cultured (10^6/mL) in 48-well plates (Costar, Cambridge, Mass) at a final volume of 0.5 mL of Iscove's Dulbecco modified medium (Biofluids, Rockville, Md) supplemented with 1% L-glutamine, 80 μg/mL gentamycin (BioWhittaker, Walkersville, Md), 1% insulin-transferrin-selenium medium (Biofluids), and 10% FCS (Hyclone, Logan, Utah) as described.[27]

PBMCs were cultured in duplicate in the presence or absence of cycloheximide (100 μg/mL; Sigma, St. Louis, Mo), human recombinant cytokines, or neutralizing antibodies to human cytokines added at various concentrations at the onset the culture. IL-4 was obtained in our laboratory by using Chinese hamster ovary cell transfectants. The human recombinant cytokines IL-6, IL-8, IL-13, and neutralizing rabbit antibodies to human IL-4, IL-13, IL-6, and TNF-α were purchased from Peprotech (Rocky Hill, NJ). Human recombinant IFN-γ and the soluble IL-4 receptor sIL-4R were obtained from Genzyme (Cambridge, Mass). Neutralizing antibody to IFN-γ was a rabbit antibody produced in our laboratory shown to completely neutralize IFN-γ activity at levels as low as 1 μg/mL. Human recombinant IL-12 was a gift from Dr S. Wolf (Genetics Institute, Cambridge, Mass). The mouse mAb to IL-4R was a gift from Dr A. Levine (Case Western Reserve University, Cleveland, Ohio). Control rabbit or mouse antisera were used where appropriate. Cultures were conducted for 10 days at 37°C with 5% CO_2. Supernatants were collected and frozen until assayed.

Assessment of IL-4 production

Cryopreserved PBMCs were thawed rapidly and washed. From approximately 50 to 100 × 10^6 cells, $CD4^+$ cells were purified by negative immunomagnetic bead selection as described previously.[28] IL-4 production was assessed at 24 hours in culture supernatants of PBMCs (2 × 10^6/mL) and purified $CD4^+$ (1 × 10^6/mL) either left unstimulated or stimulated with phorbol myristate acetate (PMA)/ionomycin as described previously.[29] IL-4 was measured by using a double sandwich ELISA as described previously.[29]

Detection of germline and productive ε-mRNA transcripts by PCR

Cryopreserved PBMCs were thawed rapidly and washed. From approximately 4 × 10^6 cells, total cellular RNA was extracted with RNAzol (Tel-Test, Friendswood, Tex). B cells (>98% purity) were purified from ~100 × 10^6 PBMCs by using immunomagnetic beads (Dynal, Lake Success, NY) as described previously,[23] and total cellular RNA was again extracted with RNAzol. To detect germline or productive ε-transcripts by PCR, cDNA was synthesized by using 2 μg of

total RNA as template, random sequence hexanucleotides as primers, and Moloney murine leukemia virus (MMLV) reverse transcriptase. Amplification of the cDNA was performed with the following primers: germline ε, 5′-ACGGAGGTGGCATTGGAGGGAATGT-3′ and 5′-AGGCTCCACTGCCCGGCACAGAAAT-3′; productive ε, 5′-GACGCTGAAGGTTTTGTT GTCG-3′ and 5′-AAGGAACC-CTGGTCACCGTCTCC-3′. The PCR was performed for 32 cycles, each cycle being 1 minute at 94°C, 1.5 minutes at 55°C, and 2 minutes at 72°C. Products were separated on a 1% agarose gel, ethidium bromide stained, and photographed.

ELISA for polyclonal IgE and IgG4

Polyclonal IgE and IgG4 were measured by ELISA as previously described.[27,30] Data are expressed in nanograms per milliliter.

Statistical analyses

Data are expressed as means ± SEM. Statistical analysis was performed with Student's *t* test for normally distributed values and the Mann-Whitney *U* test when data were not distributed normally.

RESULTS

IL-4/IL-13 dependence of elevated IgE and IgG4 production in patients with HIE

Culture supernatants of PBMCs obtained from 9 patients with HIE were assayed for the production of polyclonal IgE and IgG4 because both isotypes appear to be regulated in parallel[16] and have been shown to be elevated in this syndrome. Spontaneous levels of IgE and IgG4 produced in culture supernatants were elevated in all patients (n = 9). The geometric mean level of spontaneously produced IgE was 22.4 ng/mL (range, 8.7 to 237 ng/mL), and that of IgG4 was 6.0 ng/mL (range, 1.6 to 30.8 ng/mL). This is in marked contrast to levels in normal individuals, whose PBMCs rarely produce greater than 200 pg/mL IgE[11] and 500 pg/mL IgG4 spontaneously. As seen in Table I, the addition of exogenous recombinant human IL-4, IL-13, or the combination of IL-4 and IL-13 to individual PBMC cultures, even at elevated concentrations (100 IU/mL), was unable to alter the production of IgE or IgG4 (n = 4). IL-6, another cytokine reported to augment the production of IgG4 and IgE,[31] augmented the production of IgE slightly (18% to 41%) and IgG4 more (27% to 313%), although neither reached statistical significance.

Because IL-4 and IL-13 did not augment baseline production of IgE and IgG4 by PBMCs from patients with HIE in culture, this suggested that IgE/IgG4 production by PBMCs from patients with HIE in culture originated from B lymphocytes that were already in the process of isotype switching. To assess this more directly, germline expression of IgE was assessed in PBMCs and purified B cells from each of 2 normal individuals and 2 patients with HIE (Fig 1). As seen, both productive and germline IgE transcription could easily be detected in purified B cells of patients with HIE, whereas germline transcripts could not be detected in cells from normal individuals. Germline ε-transcripts could also be seen (albeit faintly) in the PBMC fraction of 1 patient with HIE. Productive ε-transcripts could also be detected in both PBMCs (patients with HIE only) and highly purified B cells (patients with HIE and 1 of the normal individuals).

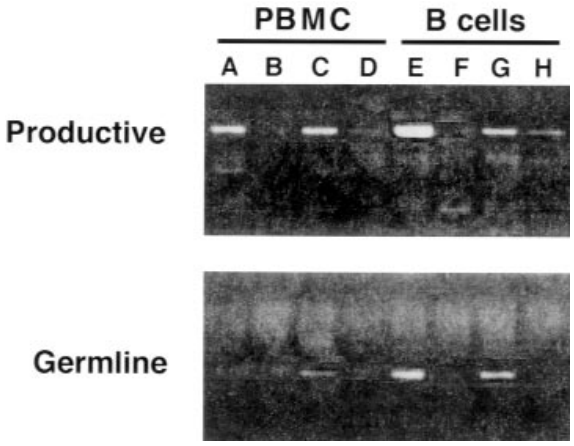

FIG 1. Productive *(top)* and germline *(bottom)* ε-transcripts detected in PBMCs (*lanes A* to *D*) and purified B cells (*lanes E* to *H*) from 2 patients with HIE (*lanes A, C, E,* and *G*) and 2 normal individuals (*lanes B, D, F,* and *H*).

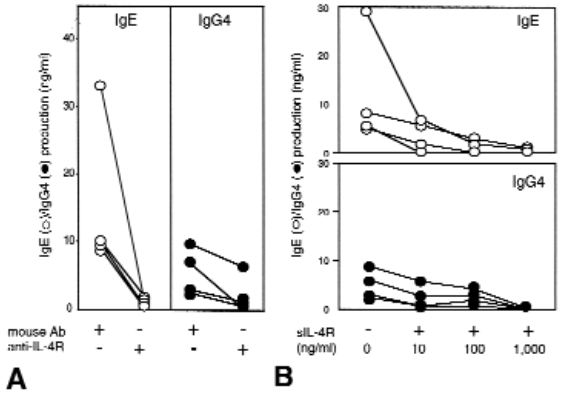

FIG 2. Antibodies to IL-4R (anti-IL-4R) or soluble IL-4R (sIL-4R) molecules can inhibit IgE/IgG4 production. IgE **(A)** or IgG4 **(B)** production in vitro in the presence of control antibody, anti-IL-4R, or sIL-4R is shown. Each set of dots represents an individual patient's response.

Neutralizing anti-IL-4 or anti-IL-13 antibodies, alone or in combination, significantly ($P < .05$; n = 8) inhibited, although they did not abolish, the production of IgE and IgG4 in vitro (Table II). In addition, in data from a limited number of experiments (n = 4), 10 μg/mL mouse mAb against human IL-4R (anti-IL-4R) dramatically reduced the production of IgE in the 4 cultures tested, and to a lesser extent, anti-IL-4R also reduced the production of IgG4 in individual PBMC cultures (Fig 2). Low concentrations (10 μg/mL) of sIL-4R reduced by up to 70% the IgE ($P < .02$) and IgG4 ($P < .01$) production in vitro, whereas more elevated concentrations (1 mg/mL) completely (IgG4) or almost completely (IgE) abolished immunoglobulin production (Fig 2). Together, these results suggest that preventing the action of IL-4 (and/or IL-13) reduced substantially the production of IgE and IgG4 in vitro in PBMCs from patients with HIE.

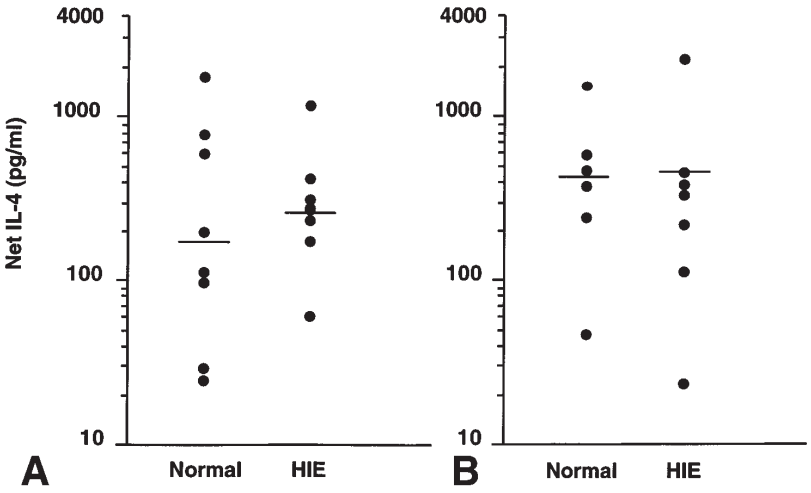

FIG 3. IL-4 production from PBMCs and purified CD4+ T cells does not differ between patients with HIE and normal individuals. Net IL-4 production (pg/mL) in PBMCs **(A)** and in purified CD4+ T cells **(B)** in response to PMA/ionomycin is shown. Each dot represents an individual subject. The *horizontal bar* represents the geometric mean.

TABLE II. The production of IgE/IgG4 in vitro by PBMCs from patients with HIE is affected but not abolished by neutralizing antibodies to IL-4 and/or IL-13*

	IgE, ng/mL (% inhibition)				IgG4, ng/mL (% inhibition)			
Patient no	Control antibody	Anti-IL-4	Anti-IL-13	Anti-IL-4 + Anti-IL-13	Control antibody	Anti-IL-4	Anti-IL-13	Anti-IL-4 + Anti-IL-13
1	20.3	3.0 (85)	14.6 (25)	4.6 (77)	5.4	0.8 (85)	1.4 (74)	0.9 (83)
2	ND	ND	ND	ND	4.4	3.8 (14)	2.1 (52)	1.6 (64)
3	16.4	8.7 (47)	13.6 (17)	7.7 (53)	5.2	9.4 (92)	0.5 (90)	0.4 (92)
4	22.1	2.6 (88)	5.3 (76)	3.9 (82)	12.6	2.6 (79)	5.7 (55)	2.8 (78)
5	24.2†	6.5 (73)	5.5 (77)	5.4 (78)	30.7	21.1 (31)	26.7 (13)	7.9 (74)
6	11.6	3.4 (71)	5.4 (53)	3.7 (86)	2.1	1.2 (43)	1.3 (38)	1.2 (43)
7	13.7	3.4 (75)	2.5 (82)	2.4 (83)	3.7	0.9 (76)	2.9 (22)	0.5 (86)
8	54.4	7.6 (86)	8.6 (84)	0 (100)	1.6	1.0 (38)	1.0 (38)	1.0 (38)
9	12.5	2.3 (82)	2.5 (80)	2.1 (83)	19.8	3.8 (81)	8.3 (58)	2.4 (88)

ND, Not done.
*Patient PBMCs (10^6/mL) were cultured for 10 days in the presence of cytokines as indicated.
†Culture supernatant derived from PBMCs from patient number 5 was diluted 1:10.

To assess whether overproduction of IL-4 (in vivo) was responsible for the elevations of IgE and IgG4 seen in HIE, spontaneous and PMA/ionomycin-driven IL-4 production was determined in PBMCs and purified $CD4^+$ T cells from the patients with HIE and from nonallergic control individuals (Fig 3). As seen, there was no difference in IL-4 levels induced by PMA/ionomycin in either PBMCs or $CD4^+$ T cells between the patients with HIE and normal individuals. In addition, there was no measurable spontaneous IL-4 production in either group of subjects (data not shown).

IL-8 and IL-6 plus TNF-α reciprocally affect the production of IgE/IgG4 by PBMCs from patients with HIE in vitro

A number of cytokines, including IL-8, have been shown to antagonize the IL-4–mediated production of IgE by B cells in normal and atopic individuals.[32,33] We thus sought to examine the role of IL-8 on the production of IgE (and IgG4) in PBMCs from patients with HIE in vitro. At low concentrations (10 IU/mL), IL-8 induced a ~70% reduction of IgE and a 50% reduction of IgG4 production, whereas more elevated concentrations (1,000 IU/mL) almost completely abolished IgGE/IgG4 production (Table III). Similarly, neutralizing antibodies to IL-6 or TNF-α consistently inhibited the production of IgE/IgG4 in individual cultures in a dose-dependent manner; the combination of neutralizing IL-6 and TNF-α abolished totally the IgE/IgG4 production (Table III).

IFN-γ and IL-12 differentially regulate IgE and IgG4 production by PBMCs from patients with HIE in vitro

Although it has been shown previously that IFN-γ is able to modulate the production of IgE in patients with HIE both in vivo and in vitro,[11] the effect of IL-12 (a potent inducer of IFN-γ that can also affect B-cell differ-

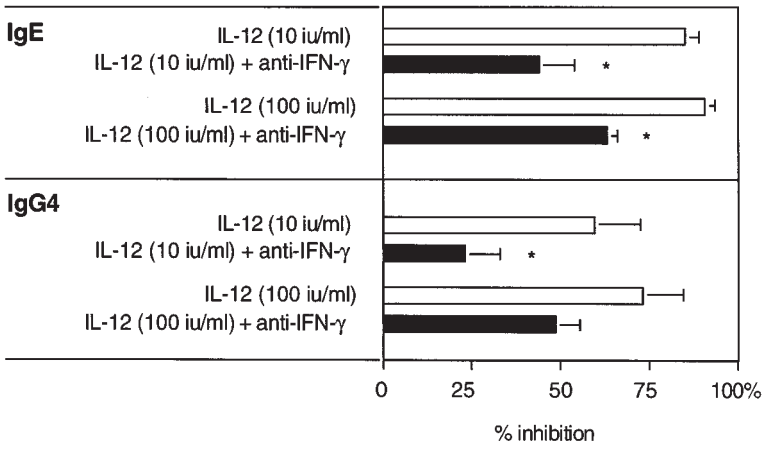

FIG 4. IL-12 affects IgE **(A)** and IgG4 **(B)** production in both an IFN-γ–dependent and an IFN-γ–independent manner. Mean ± SEM of percent inhibition in IgE/IgG4 production over spontaneous production in the presence or absence of neutralizing anti-IFN-γ antibodies is shown. *Asterisks* indicate statistical significance (*$P < .05$ between controls and cultures with anti-IFN-γ antibodies).

entiation) on IgE and IgG4 production was assessed and compared directly with that of IFN-γ (Table IV).

As little as 10 IU/mL of either of the cytokines was sufficient to diminish spontaneous IgE production by approximately 60% to 80% ($P < .01$; n = 9). Increasing the concentration 100-fold only gave a modest increase (from 80% to 95%) in suppression of IgE ($P < .05$ compared with 10 IU/mL). IL-12 was significantly more efficient than IFN-γ at low concentrations (10 IU/mL) in diminishing the production of IgG4 ($P < .05$). Of note, IL-12 was still more inhibitory than IFN-γ on IgG4 production in vitro when used at a concentration of 100 IU/mL, although not significantly. At more elevated concentrations of IL-12 (1000 or 10,000 IU/mL), the marked inhibitory effect on IgG4 production was lost to a small but significant degree ($P = .015$), whereas similar concentrations of IFN-γ were still inhibitory.

To examine whether IL-12 affected IgE and/or IgG4 production by PBMCs from patients with HIE directly or indirectly through the induction of IFN-γ, individual cultures were performed in the presence of either IL-12 or IL-12 plus 20 μg/mL neutralizing rabbit anti-IFN-γ. As can be seen (Fig 4), at low concentrations of IL-12, approximately 50% of the downregulatory effect of IL-12 on both IgE and IgG4 production was attributable to IFN-γ because anti-IFN-γ antibody significantly reduced the IL-12-mediated inhibitory effect ($P < .05$; n = 8). This effect was lost in a dose-dependent manner with increasing concentrations of IL-12 in individual cultures.

DISCUSSION

In HIE (Job's) syndrome, the observation that PBMCs were capable of secreting substantial levels of IgG4[11,34,35] along with IgE[11] strongly suggested that an IL-4/IL-13-mediated pathway was implicated. Indeed, IL-4 and IL-13 are the only cytokines capable of inducing the switching from μ to either γ4 or ε at the genomic level in human B cells,[15] a process that can be counteracted by IFN-γ[36,37] and IL-12.[38,39] IFN-γ and IL-12 are therefore considered natural antagonists of IL-4 (and likely IL-13).[40] We have thus targeted both the inductive (IL-4/IL-13) and suppressive (IFN-γ/IL-12) arms of the IgE/IgG4 response for our studies.

This study demonstrates that IgE production by PBMCs from patients with HIE was not augmented when exogenous IL-4 was provided, corroborating previously published results.[41] In the presence of neutralizing antibodies to IL-4 and/or IL-13, although IgE and IgG4 were inhibited significantly but not completely, similar to the level of inhibition observed in atopic individuals,[42] mAbs to the IL-4R and the soluble form of the IL-4R (sIL-4R) were capable of completely abolishing IgE/IgG4 production in vitro. These data suggest that patients with HIE have an IL-4/IL-13–mediated expansion of IgE- and IgG4-producing B cells, an expansion that can be modulated by blocking the interaction of IL-4/IL-13 with their common receptor.[43]

IL-4 and IL-13 have been shown to induce switching in naive B cells in preference to expanding predifferentiated B cells as shown in healthy donors[16,18,37] or filaria-infected individuals.[23] Because IL-4 and IL-13 did not augment baseline production of IgE and IgG4 by PBMCs from patients with HIE in culture, it can be deduced that B cells produce these isotypes at near maximum levels. This would imply that IgE/IgG4 production by PBMCs from patients with HIE in culture originated from B lymphocytes that had already switched to γ4 or ε, suggested by preliminary evidence that germline expression of IgE (Fig 1) and IgG4 (not shown) could easily be detected in B cells from patients with HIE and not from normal individuals.

IL-6 was shown to augment (albeit slightly) IgG4 production in PBMCs from patients with HIE. IL-6 has been shown to act at different levels in B-cell differentiation: it synergizes with IL-4 to induce IgE production, and it also expands preswitched (primarily $s\gamma^+$) B lymphocytes to produce IgG.[31,44-46] In this study IL-6 likely expanded preswitched $sIgG4^+$ B cells. Although it is not considered a B-cell differentiation factor stricto sensu,[45] IL-6 is

TABLE III. The production of IgE/IgG4 in vitro by PBMCs from patients with HIE is affected by neutralizing antibodies to human IL-6 and to TNF-α*

	IgE, ng/mL (% inhibition)						IgG4†, ng/mL (% inhibition)					
Patient no	Control antibody	Anti-IL-6	Anti-TNF-α	Anti-IL-6 + Anti-TNF-α	Control (medium)	IL-8‡	Control antibody	Anti-IL-6	Anti-TNF-α	Anti-IL-6+ Anti-TNF-α	Control (medium)	IL-8‡
1	5.7	1.2 (79)	1.4 (75)	0 (100)	5.6	1.4 (75)	4.5	0.3 (93)	0.5 (89)	0 (100)	2.3	0.5 (81)
2	5.1	0 (100)	2.2 (57)	0 (100)	5.0	0 (100)	7.5	0.6 (92)	2.6 (65)	0 (100)	6	0 (100)
3	29.3	1.1 (96)	7.1 (76)	0 (100)	28.3	27 (5)	2.5	0 (100)	1.1 (56)	0 (100)	8.8	3.4 (61)
4	ND	ND	ND	ND	8.4	0 (100)	ND	ND	ND	ND	2.9	0.5 (83)
5	6.7	0.2 (97)	2.1 (69)	0 (100)	ND	ND	2.9	0.2 (93)	1.1 (62)	0 (100)	ND	ND

ND, not done.
*Patient PBMCs (10^6/mL) were cultured for 10 days in the presence of 10 μg/mL neutralizing or control antibody.
†IL-8 experiments for IgG4 were done separately by using thawed PBMCs from the same donors.
‡Recombinant human IL-8 was used at various concentrations; shown are values for 100 IU/mL.

TABLE IV. The production of IgE/IgG4 in vitro by patients with HIE is differently affected by human recombinant IFN-γ and IL-12

	Ig production, ng/mL (% inhibition)							
Culture conditions*	Patient 1	Patient 2	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9
IgE (dilution 1:5)								
Spontaneous	ND	5.5	24.4	32.9	3.6	3.6	14.6	5.3
Cycloheximide (100 μg/mL)	ND	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)
IFN-γ (10 IU/mL)	ND	1.1 (80)	2.1 (91)	16.9 (49)	1.3 (64)	1 (72)	11 (26)	0 (100)
IFN-γ (100 IU/mL)	ND	0.9 (84)	0.5 (98)	7.1 (78)	0.2 (94)	0 (100)	0 (100)	0.3 (94)
IFN-γ (1000 IU/mL)	ND	0 (100)	0 (100)	4.1 (88)	0 (100)	0 (100)	0 (100)	0 (100)
IFN-γ (10,000 IU/mL)	ND	0 (100)	0 (100)	2.3 (93)	0 (100)	0 (100)	0 (100)	0 (100)
IL-12 (10 IU/mL)	ND	1.1 (80)	1.1 (95)	1.4 (96)	1 (72)	0.8 (78)	1.3 (91)	1.2 (75)
IL-12 (100 IU/mL)	ND	0.9 (84)	0.7 (97)	2.3 (93)	0.8 (78)	0.7 (81)	1.2 (92)	0.8 (85)
IL-12 (1000 IU/mL)	ND	0.6 (89)	0.7 (97)	6.9 (79)	0.5 (86)	0.5 (86)	1.3 (91)	0.3 (94)
IL-12 (10,000 IU/mL)	ND	4 (9)	0.6 (98)	0.7 (98)	0 (100)	0.2 (94)	1.5 (90)	0.5 (91)
IgG4 (undiluted)								
Spontaneous	6.4	3.9	10.1	30.8	2.7	3.9	1.6	22.7
Cycloheximide (100 μg/mL)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)
IFN-γ (10 IU/mL)	3.5 (45)	3 (23)	3.6 (64)	27.3 (11)	2.5 (7)	1.2 (69)	1.1 (31)	12.5 (44)
IFN-γ (100 IU/mL)	3.2 (50)	2.8 (28)	3.5 (65)	22.1 (28)	2.3 (15)	0.6 (85)	0.6 (62)	11.7 (48)
IFN-γ (1000 IU/mL)	2.1 (67)	2.6 (33)	3.1 (69)	17.1 (44)	1.5 (44)	1.4 (64)	0 (100)	3.2 (86)
IFN-γ (10,000 IU/mL)	2.1 (67)	1.9 (51)	1.7 (83)	22.6 (27)	1.4 (48)	3.3 (15)	0 (100)	6.4 (72)
IL-12 (10 IU/mL)	1.5 (76)	1.6 (59)	9.1 (10)	21.5 (30)	0.9 (67)	0.9 (77)	0.4 (75)	0.9 (96)
IL-12 (100 IU/mL)	1.4 (78)	1.4 (64)	5.9 (41)	18.9 (39)	0.9 (67)	0 (100)	0 (100)	1.2 (95)
IL-12 (1000 IU/mL)	2.9 (55)	3.4 (13)	4.8 (52)	14.9 (53)	1.7 (37)	0.8 (79)	0 (100)	2.2 (90)
IL-12 (10,000 IU/mL)	6.1 (5)	3.9 (0)	6.9 (32)	23.9 (22)	1.9 (30)	1.5 (61)	0.2 (92)	9.6 (58)

ND, Not done.
*Patient PBMCs (10^6/mL) were cultured for 10 days in the presence of cytokines as indicated.

known to synergize with IL-4 in enhancing the production of IgE.[31,46] Indeed, IL-6 acts in both an autocrine and paracrine manner,[47] as does TNF-α,[47] a pleiotropic cytokine that is also capable of enhancing IL-4–induced IgE secretion by normal B cells.[48]

IL-8, a cytokine that has been described as an IL-4 antagonist that acts independent of IFN-α and IFN-γ,[32,33,49] has proven capable of inhibiting the spontaneous production of IgE/IgG4 in atopic individuals (by affecting $sIgE^+$ and $sIgG4^+$ B lymphocytes) by inhibiting the endogenous production of IL-6 and TNF-α by committed B cells.[33] In this study exogenous IL-8 was capable of abolishing the production of IgE/IgG4 by PBMCs from patients with HIE, even at very low concentrations (10 IU/mL). Furthermore, neutralizing anti-IL-6 and anti-TNF-α antibodies together completely abolished IgE (and IgG4) production in PBMCs from patients with HIE, a finding having parallels in IgE/IgG4 production in parasite-infected patients.[23] Collectively, our data would suggest that overproduction of IgE/IgG4 by PBMCs from patients with HIE derives from B cells that have been differentiated in vivo before the culture and that were being terminally differentiated in the presence of maturing factors (probably IL-4/IL-13) released by PBMCs in culture.

This would imply that IL-4 and IL-13 likely originate from non-B-cell sources because these cytokines are not detected generally in either normal or leukemic B cells.[49-51] Cytokines, such as IL-8 and antagonists of IL-6 and TNF-α, all of which are capable of affecting both the differentiated B cells and the IL-4–induced IgE production, would thus be particularly relevant in downregulating such IgE/IgG4 production in patients with HIE.

Taken together, our data seem to indicate a profound impairment in the regulators of IL-4–dependent IgE production in HIE, including the IL-4/IL-4R pathway itself. Indeed, as described recently,[12] 3 patients with HIE were found to be heterozygous for the Q576R allele of the IL-4R α-chain, which appears to allow for a gain in IL-4–mediated intracellular signaling and function (as assessed by CD23 upregulation). Of interest, only 3 of the patients in the present study are heterozygous for this mutant allele (data not shown), and the frequency of this allele in patients with HIE is no different from that in the population at large.[13]

A previous study demonstrated that recombinant human IFN-γ was capable of diminishing the production of IgE (and IgG1, IgG3, and IgG4) in patients with HIE both in vivo and in vitro.[11] The present study confirms and extends these findings, showing that more elevated concentrations of IFN-γ could diminish but not abolish IgE (and IgG4) production in vitro. IFN-γ has already been used as a cytokine-based alternative therapy in Job's and Omenn's syndromes.[52] We have shown, in addition, that IL-12 could affect spontaneous IgE/IgG4 production in a manner comparable with that of IFN-γ. An interesting feature is that IL-12, at concentrations 10-fold lower than that of IFN-γ, can inhibit IgG4 production in HIE. IL-12 affected IgE/IgG4 production in vitro in both an IFN-γ–dependent and an IFN-γ–independent manner because neutralizing antibodies to IFN-γ failed to account for the total effect of IL-12. IL-12 has recently been reported to suppress IgE and to enhance IgG4 production by PBMCs in normal individuals, although this has not been examined at a dose-dependent level.[24] Results presented here clearly demonstrate that IL-12 differentially regulates the production of IgG4 in vitro, depending on its concentration.

There is a need for novel approaches to the management of diseases with extreme elevations of IgE associated with pathologic consequences (eg, allergic diatheses, nephrotic syndrome, and certain immunodeficiency syndromes).[35,53,54] To this end, our findings suggest that IFN-γ is still a cytokine of interest in the treatment of HIE but that IL-12 might represent a less toxic and alternative therapy. Moreover, therapies inhibiting the binding of IL-4/IL-13 to its receptor might represent a major new advance in the treatment of HIE and other conditions associated with extreme IgE states.

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