

Th1/Th2 cytokine imbalance in a family with hyper-IgE syndrome

M.G. Netea^{1,4,*}, P.M. Schneeberger², E. de Vries³, B.-J. Kullberg⁴,
J.W.M. van der Meer^{4,**}, M.I. Koolen¹

Departments of ¹Internal Medicine, ²Medical Microbiology, and ³Paediatrics, Bosch MediCentrum, 's-Hertogenbosch, the Netherlands, ⁴Department of Medicine (541), University Medical Centre St Radboud, PO Box 9101, 6500 HB Nijmegen, the Netherlands, tel.: +31 (0)24-361 88 19, fax: +31 (0)24-354 17 34, * corresponding author

ABSTRACT

Background: Hyperimmunoglobulin E (hyper-IgE) syndrome is a rare immunodeficiency characterised by recurrent skin and respiratory tract infections, skeletal and dental abnormalities, chronic eczema, and elevated serum IgE. We describe a family with four hyper-IgE syndrome patients (38, 37, 30 and 7 years old), in which we investigated the cytokine response to both specific and non-specific stimulation.

Methods: Whole blood from patients and volunteers was stimulated for either 24 or 48h at 37°C with heat-killed *Staphylococcus*, *C. albicans* or a combination of IL-12 and IL-18. Cytokine concentrations in the plasma were measured by specific radioimmuno-assays or ELISA.

Results: Serum IgE ranged from 5000 to 16,670 IU/ml, and neutrophil chemotaxis was normal in all four patients. Tumour necrosis factor, interleukin (IL)-1 β , IL-6 and IL-8 production after stimulation of whole-blood cultures with lipopolysaccharide or heat-killed *S. aureus* did not differ between the adult patients and four healthy controls. In contrast, when blood from patients and controls was stimulated with heat-killed *S. aureus* or *C. albicans*, a severe imbalance towards a Th2 phenotype was found, with 10- to 30-fold reduction in the IFN γ /IL-10 ratios in the hyper-IgE syndrome patients. The IFN γ production in the patients was less severely impaired when blood was non-specifically stimulated with a combination of IL-18 and IL-12.

Conclusion: In this family with hyper-IgE syndrome, the imbalance in the Th1/Th2 cytokine production may have

been involved in the pathogenesis of the recurrent infections and/or chronic eczema characteristic of this disease.

INTRODUCTION

Hyperimmunoglobulin E syndrome is a rare immunodeficiency characterised by recurrent staphylococcal skin abscesses, respiratory tract infections such as pneumonia with pneumatocele formation, mucocutaneous candidiasis, chronic eczematous dermatitis, and extremely elevated circulating IgE concentrations.^{1,2} The recent publication of the clinical characteristics of a large series of patients revealed important non-immunological features such as dental abnormalities (retained primary teeth, non-eruption of permanent teeth, double rows of teeth), anomalies in midline facial development, and skeletal abnormalities (bone fractures, hyperextensible joints, scoliosis).³ Most cases are sporadic, but the description of several families with clustering of hyper-IgE syndrome patients suggests an autosomal dominant transmission with variable expressivity.³ Although the precise genetic abnormality is not yet known, linkage of hyper-IgE syndrome to the proximal q region of the chromosome 4 has been demonstrated.⁴

It has been hypothesised that the increased susceptibility to infections and the skin hypersensitivity could be the result of an altered cytokine production pattern in hyper-IgE syndrome patients. IFN γ as a prototype of the Th1 cytokines activates the cellular immune responses, whereas IL-4 and IL-10 as cytokines of the Th2 family induce activation of the

** J.W.M. van der Meer was not involved in the handling and review process of this paper.

humoral immune system.⁵ A bias towards Th1 responses is associated with autoimmune diseases, whereas a Th2 imbalance induces allergic reactions through hypereosinophilia and hyper-IgE production. A defective IL-12/IFN γ axis leads to high susceptibility to infections.⁶ Several studies have tried to document a defective production of IFN γ by cells from hyper-IgE syndrome patients, with variable results.⁷⁻¹¹ However, these studies have used mitogenic stimulation with phytohaemagglutinin (PHA), and not more relevant stimuli derived from microbes for which the hyper-IgE syndrome patients are highly susceptible. In a recent study, Borges and colleagues used *Staphylococcus aureus* as a cytokine stimulus, and they reported a defective IL-12/IFN γ pathway in patients with hyper-IgE syndrome.¹² Very limited information is available regarding production of Th2 cytokines by cells of hyper-IgE syndrome patients, with a production of IL-4 upon PHA stimulation which is not altered compared with controls.^{7,11}

In the present study we investigated the Th1/Th2 cytokine balance in three adult patients with hyper-IgE syndrome by using whole-blood stimulation with either heat-killed *S. aureus* or *C. albicans*. The patients belong to a family in which another seven-year-old patient was diagnosed, and in which five other individuals also have clinical and/or laboratory abnormalities suggestive of hyper-IgE syndrome. In addition, we also have studied the capacity of the blood collected from the patients to produce proinflammatory cytokines such as TNF, IL-1 β , IL-6 and the chemokine IL-8.

PATIENTS AND METHODS

Patients

One patient under control for essential hypertension at the outpatient clinic of Bosch MediCentrum, 's-Hertogenbosch, reported recurrent skin abscesses, recurrent respiratory tract infections since childhood (but no pneumatocele or bronchiectasis), and chronic eczematous dermatitis. From the family history it became apparent that the patient's brother and sister, as well as a nephew, had similar clinical manifestations (figure 1). Other manifestations in the four patients included chronic candidiasis of the nail beds (all patients), late loss of primary teeth (in two of them), and skeletal abnormalities (one patient cheilopalatoschisis, and one patient three fractures of the left wrist). The characteristic coarse facies was present in a mild form in two of the patients. When the scoring system proposed by Grimbacher *et al.*⁴ was used, all these four individuals scored higher than 14 (17, 23, 24 and 33), consistent with the diagnosis of hyper-IgE syndrome. In addition, five other members of the family scored between 10 and 14, being classified as 'unknown' patients (figure 1). The deceased mother of the three adult patients had severe recurrent skin abscesses and it was recalled to be said that 'she was

like Job'. It is of note that hyper-IgE syndrome used to be known as Job's syndrome.¹ The other 'unknown' patients had features of chronic eczema, recurrent skin infections, and moderately increased IgE concentrations (in two of them). The cause of death of patients I.1 and II.1 was malignancy and a ruptured aortic aneurysm, respectively. All hyper-IgE syndrome patients were evaluated during an infection-free interval. Informed consent was obtained from all subjects before the studies were performed.

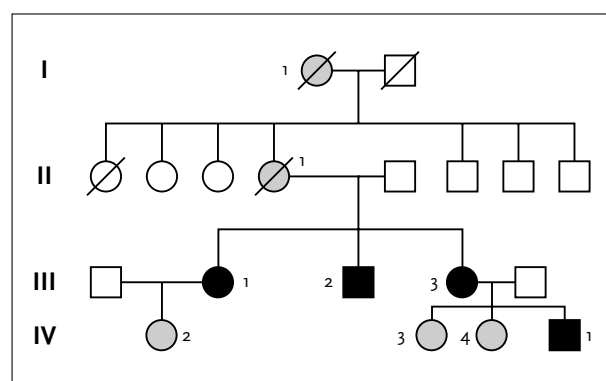


Figure 1
Pedigree of a family with hyper-IgE syndrome

The squares represent male family members, and the circles represent female family members. A slash indicates that a person has died. Open symbols represent healthy persons, closed symbols represent patients with hyper-IgE syndrome and hatched symbols represent persons with clinical and/or laboratory characteristics associated with hyper-IgE syndrome and uncommon in the general population. Generations are labelled with Roman numerals and patients with Latin numerals.

Cytokine studies

The three adult patients (aged 27, 30 and 38 years) and four normal subjects (two men and two women), without atopic diseases or chronic infectious complications, ranging in age from 26 to 47 years, were investigated as study and control groups for cytokine production.

Blood was collected by venous puncture from the cubital vein in heparinised tubes (Monoject, 's-Hertogenbosch, the Netherlands). Whole-blood cultures were performed as described previously.¹³ Briefly, 200 μ l blood was added to 100 μ l stimulus and 700 μ l RPMI 1640, and incubated in 24-wells plates (Greiner, Alphen aan den Rijn, the Netherlands) at 37°C. As stimuli we used 10⁷ CFU/ml heat-killed (30 minutes at 100°C) *S. aureus* (clinical isolate from the patients) or *C. albicans* (ATCC 10231), or 10 ng/ml lipopolysaccharide (LPS, *E.coli* serotype O55:B5, Sigma, St. Louis), or a combination of 1 ng/ml rhIL12 (Peprotech) + 10 ng/ml rhIL-18 (kindly provided by Dr. Masashi Kurimoto, Hayashibara Laboratories, Okayama, Japan). The plasma was isolated for 24 hours (for TNF, IL-1 β , IL-6 and IL-8 measurements) or 48 hours (for IFN γ and IL-10

determinations) after incubation, and the samples were kept at -70°C until cytokine measurements had been completed.

TNF α and IL-1 β concentrations were determined by specific radioimmunoassays as described.¹⁴ IL-6, IL-8, IL-10 and IFN γ were measured by commercial ELISA kits (Pelikine Compact, CLB, Amsterdam, the Netherlands), according to the instructions of the manufacturer.

RESULTS

Immunological characteristics

The laboratory characteristics of the patients are presented in *table 1*. The patients had normal leucocyte and neutrophil counts, with normal CD4, CD8 and CD4/CD8 ratios. Chemotactic response of the neutrophils towards IL-8 was approximately 60% of the normal in two patients, and almost normal in the other two. Similar data were obtained when chemotaxis towards C5a or PAF was performed (not shown). The NK-cell population was in the low-normal range, and the B-cell population was normal. Complement factors C3 and C4 were normal, and Ig fractions A, M, and G were present in normal concentrations. Eosinophil counts ranged from 250 to 900 eosinophils/ μ l, and the serum IgE concentrations from 5000 to 16,700 UI/ml. *S. aureus* was isolated from skin abscesses and nose of all four patients, and after binary strain typing¹⁵ it appeared that they all had the same strain.

IFN γ and IL-10 production

Stimulation of whole blood from hyper-IgE syndrome patients with heat-killed *S. aureus* led to 13- to 36-fold

lower IFN γ production compared with control volunteers (*table 2*). Similarly, *C. albicans* stimulation induced 16- to 180-fold lower IFN γ synthesis in blood cultures of hyper-IgE syndrome patients compared with controls. In contrast, production of IL-10 was in the same range in the hyper-IgE syndrome patients and the healthy volunteers. IFN γ /IL-10 ratios were severely reduced in the patients compared with controls (*table 2*). IL-12 and IL-18 synergistically stimulates T cells for the production of IFN γ . When whole blood from hyper-IgE syndrome patients was stimulated with a combination of IL-12 +IL-18, 1.5 to 9-fold less IFN γ was produced compared with the blood from healthy controls (*table 2*). As expected, no IL-10 was produced in response to IL-12/IL-18 stimulation (all samples below detection limit).

Production of the proinflammatory cytokines TNF, IL-1 β , IL-6 and IL-8

When whole blood from hyper-IgE syndrome patients was stimulated with either *S. aureus* (*table 3*) or *C. albicans* (not shown), no differences compared with control volunteers could be found. Stimulation of blood with a non-related stimulus, the LPS component of Gram-negative bacteria, also led to a similar proinflammatory cytokine profile in patients and healthy subjects (*table 3*).

DISCUSSION

The results of the investigations we performed suggest that patients with hyper-IgE syndrome have a strong bias towards a Th2 response, expressed as reduced IFN γ /IL10 ratios, especially when specifically stimulated with heat-

Table 1
Clinical and laboratory characteristics of four patients with hyper-IgE syndrome

	PATIENT III.1	PATIENT III.2	PATIENT III.3	PATIENT IV.1	NORMAL
Age (years)	27	30	38	7	
Sex	Female	Female	Male	Male	
Leucocytes (x 10 ⁹ /l)	8.3	6.2	7.4	9.7	4.3-10.8
Lymphocytes (x 10 ⁹ /l)	2.1	1.7	1.7	2.9	1.0-2.9
CD3+ (x 10 ⁹ /l)	1.7	1.3	1.3	2.2	0.7-2.1
CD3+CD4+ (x 10 ⁹ /l)	1.1	0.9	0.9	1.2	0.3-1.4
CD3+CD8+ (x 10 ⁹ /l)	0.6	0.4	0.4	0.8	0.2-0.9
CD3-CD16+CD56+(x 10 ⁹ /l)	0.08	0.12	0.09	0.1	0.09-0.6
CD19+ (x 10 ⁹ /l)	0.2	0.2	0.2	0.5	0.1-0.5
PMN chemotaxis (% norm)	88	55	89	62	100
C3 (g/l)	1.5	1.5	1.0	1.6	0.8-1.7
C4 (g/l)	0.27	0.31	0.29	0.42	0.14-0.45
IgG (g/l)	10	12	10	10	6-15
IgA (g/l)	0.9	2.3	1.7	0.8	0.7-4.6
IgM (g/l)	1.4	1.5	1.1	0.7	0.3-2.3
IgE (UI/ml)	7210	12,140	16,700	5000	<150
Eosinophils (x 10 ⁹ /l)	410	900	500	250	50-350

Table 2

*IFN γ and IL-10 production in hyper-IgE syndrome patients and healthy controls after stimulation of whole-blood cultures with either heat-killed *S. aureus*, *C. albicans* or a combination of IL-12 + IL-18*

		PATIENT III.1	PATIENT III.2	PATIENT III.3	CONTROLS*
IFN γ (pg/ml)	<i>S. aureus</i>	150	290	230	3895 $\pm$ 1228
	<i>C. albicans</i>	20	225	37	3697 $\pm$ 1046
	IL-12/IL-18	130	640	97	953 $\pm$ 329
IL-10 (pg/ml)	<i>S. aureus</i>	129	159	441	210 $\pm$ 97
	<i>C. albicans</i>	306	228	96	97 $\pm$ 32
	IL-12/IL-18	<5	<5	<5	<5
IFN γ /IL-10	<i>S. aureus</i>	1.2	1.8	0.5	21.3 $\pm$ 8.4
	<i>C. albicans</i>	0.07	1.0	0.4	36.3 $\pm$ 9.1

* Mean $\pm$ standard deviation of four volunteers.

Table 3

*Proinflammatory cytokine production in hyper-IgE syndrome patients and healthy controls after stimulation of whole-blood cultures with either heat-killed *S. aureus* or LPS*

		PATIENT III.1	PATIENT III.2	PATIENT III.3	CONTROLS*
TNF (ng/ml)	<i>S. aureus</i>	8.2	6.6	7.6	7.7 $\pm$ 2.4
	LPS	0.9	3.6	2.7	2.6 $\pm$ 1.1
IL-1 β (ng/ml)	<i>S. aureus</i>	6.3	7.2	9.1	6.7 $\pm$ 2.1
	LPS	0.4	1.7	2.1	0.9 $\pm$ 0.3
IL-6 (ng/ml)	<i>S. aureus</i>	24.8	7.6	17.8	12.9 $\pm$ 3.1
	LPS	6.2	12.5	17.2	10.5 $\pm$ 2.7
IL-8 (ng/ml)	<i>S. aureus</i>	153	31	77	47.5 $\pm$ 9.2
	LPS	7.7	8.1	13.1	8.8 $\pm$ 1.8

* Mean $\pm$ standard deviation of four volunteers.

killed *S. aureus* or *C. albicans*. Whereas Th1 cytokines are important for stimulating the defence against intracellular micro-organisms, Th2 cytokines activate the humoral arm of the immune system and are involved in the defence against parasites and protozoa.⁵ The recurrent infections of the skin and the respiratory tract in patients with hyper-IgE syndrome fit a Th1/Th2 imbalance, with low IFN γ production. In contrast, relatively high IL-4 and IL-10 concentrations could explain the high IgE concentrations, eosinophilia and allergic reactions. Our data are sustained by the study by Borges *et al.*, who also observed impaired IFN γ production in cells isolated from hyper-IgE syndrome patients stimulated with *S. aureus*.¹² Although the IFN γ /IL-10 ratios were strongly reduced, it is remarkable that IL-10 concentrations were normal.

IFN γ is mainly produced by T cells and NK cells, and its synthesis can be induced through different mechanisms. Firstly, an antigen can stimulate a monocyte/macrophage to produce proinflammatory cytokines such as IL-12, IL-18, IL-23 and to a lesser extent IL-15 or IL-1 β , which in turn stimulate IFN γ synthesis from T cells and NK cells.^{16,17} Secondly, T cells can be directly stimulated to produce IFN γ either by mitogens, or by components of bacteria directly binding to the T-cell receptor and acting as superantigens.¹⁸

Finally, when antigen-specific T cells bind the antigen presented by the MHC complex, they also respond with IFN γ production. When the blood of hyper-IgE syndrome patients was stimulated with heat-killed *S. aureus* or *C. albicans*, the production of IFN γ was lower compared with controls. Because stimulation with a combination of IL-12/IL-18 (which can directly stimulate T cells) also led to a lower IFN γ production in the hyper-IgE syndrome patients, it is likely that the defect leading to this skewed Th1/Th2 balance is located at the level of T cells, and not the monocytes/macrophages. This conclusion is sustained by the normal monocyte function as expressed by normal production of TNF, IL-1 β , IL-6 and IL-8 in our study, and of normal IL-12 synthesis observed by others.^{11,12} Chehimi and colleagues have demonstrated a decreased IL-12R β 2 expression in cells from hyper-IgE syndrome patients,¹¹ which could explain the decreased response upon IL-12/IL-18 stimulation in our study. Another argument in favour of a T-cell defect in hyper-IgE syndrome patients comes from studies showing a defective IFN γ synthesis upon mitogenic stimulation with PHA.^{7,8,10} In addition, Rodriguez and colleagues observed higher IL-4 production in cells of hyper-IgE syndrome patients compared with controls, which would also argue for a Th2 bias.⁹ We could not confirm this, however.

No defect in neutrophil chemotaxis was found in two of our patients, whereas in two others a 40% decreased chemotaxis was measured when IL-8, C5a or PAF were used as chemoattractants. The chemotactic defect in the syndrome is a controversial issue. Some reports show a marked defect in neutrophil chemotaxis in hyper-IgE syndrome patients, whereas others do not.^{19,20} It has been proposed that an increased production of granulocyte-macrophage colony-stimulating factor might be responsible for this effect.²⁰ The reason for the discrepancy between these data is not clear. No other immune abnormalities, in terms of lymphocyte numbers and phenotype, NK-cell numbers, complement factors, immunoglobulin classes (except of course IgE), have been found in our patients.

In conclusion, hyper-IgE syndrome is a complex multisystem disease with both immunological and non-immunological abnormalities. Although our study investigated a small number of patients, all from one affected family, the data obtained strongly suggest that the increased susceptibility to infections and the chronic allergic dermatitis may be the result of an altered Th1/Th2 cytokine profile towards a Th2 bias. We are currently performing a larger study of hyper-IgE syndrome patients to try to confirm and extend the data presented here. These findings could have important therapeutic implications, as it has been shown that recombinant IFN γ can enhance neutrophil chemotactic responses in hyper-IgE syndrome patients²¹ and a small pilot study has suggested beneficial effects of treatment with recombinant IFN γ in these patients.²²

ACKNOWLEDGEMENT

We want to thank Dr. T. Kuijpers (CLB, Amsterdam) for the help with the chemotaxis assay, and L. Jacobs and T. Verver-Jansen for performing the cytokine determinations.

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