Interferon-gamma Response to Various Stimuli in Hyperimmunoglobulin E Syndrome

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Hyper-IgE syndrome, also known as Job's syndrome, is a rare immunodeficiency disorder clinically characterized by recurrent infections of the skin, with formation of deep-seated abscesses, as well as by upper and lower respiratory tract infections. The most remarkable diagnostic finding is the increased levels of IgE antibodies to levels higher than those detected in other diseases like asthma, parasitic infections and others. One of the pathogenetic mechanisms suggested focuses on a deficiency or a decrease in the ability of T suppressor cells to control overactive T helper cells type two (Th2). The latter produce cytokines such as IL-4, which stimulate B cells to switch to IgE production. Th1 type of cells which produce several cytokines necessary to ensure an adequate response to infections such as neutrophil phagocytosis are consequently less active, and thus the level of production of one of their cytokines, Interferon gamma (IFNγ), in response to other stimuli had been used as a marker of their activity (1). In our study, we compared IFNγ production in a patient with Job's syndrome to that of his father and mother. An autosomal mode of inheritance was suspected in Job's syndrome. Induction of INFγ by various antigens was studied in all three individuals. The results showed the overall absence of response in the son (the patient) to that of his father and mother in both whole peripheral blood and peripheral blood mononuclear cell samples. Furthermore, the overall response levels of IFNγ showed more similarities between the patient and his mother. These results lend further support to the above-suggested pathogenetic mechanism in Job's syndrome and may show inheritance of the disease from the mother's side.

Key words: hyper IgE syndrome, immunoglobulin E, interferon gamma, T helper1, T helper 2, T suppressor

INTRODUCTION

Hyper-immunoglobulin E syndrome (HIES), also known as Job's syndrome, is a disease of not yet clear aetiology which is characterized by recurrent infections since early childhood, involving skin, as well as the upper and lower respiratory tract. However, any organ can be affected (2–5). The most distinctive objective finding in all (more than 200) reported cases was highly increased immunoglobulin class E (IgE) serum levels. Fluctuations of serum IgE among different patients and through time in the same patient vary a lot, and thus a wide range has been observed roughly extending between values as low as 900 to those higher than 58,000 IU/ml. Eosinophilia is another frequently observed characteristic symptom of the HIES (6, 7).

The medical history of every patient reported thus far showed at least a single episode of severe elevation of IgE levels in serum, i.e. a peak level greater than 2000 IU/ml. Even normal IgE levels in an adult are not sufficient to exclude Job's syndrome if other diseases have been excluded (8).

One of the pathogenetic mechanisms involved in HIES is the absence of normal functioning of T suppressor cells, overactive T helper type two (Th2) cells, and loss of sufficient cytokine production by Th1 cells which participate in regulating normal immune reactions to infections. The aim of our study was to test one aspect of the above theory, mainly that of analyzing IFNγ production by the monocy-

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tes of a young patient diagnosed with HIES. Our second aim was to detect indirectly any possible inheritance pattern by comparing the immunologic response in three members of our patient's family.

MATERIALS AND METHODS

We tested IFNγ response to various stimuli in whole peripheral blood and peripheral blood mononuclear cell samples taken from the first patient in Latvia diagnosed with HIES. The results did reveal a more pronounced absence of proper response in the patient's specimens that further consolidated the clinical picture of a HIES.

Mr. E., a twenty-one-year-old male patient with a history since early hildhood of recurrent infections involving skin, salivary glands, upper respiratory system and gastrointestinal tract, splenomegaly and lymphadenopathy, was admitted to our clinic due to some abdominal pain increased temperature and some night sweating. He also had eosinophilia (13%). It's worth mentioning that allergic manifestations are a frequent problem in HIES and eosinophilia is thought to be due to the stimulatory effect of IL-5 produced by overactive Th2 cells on eosinophils. Other laboratory findings included monocytosis (12%) and increased IgE levels (1120 IU/ml). Other biochemistry, liver enzymes, and urine analysis were normal. Further investigations excluded the presence of food allergies, tuberculosis, and parasitic infections. Additionally, cytomegalovirus (CMV) IgM, Epstein–Barr virus (EBV) IgG as well as hepatitis B surface antigen (HBsAg) and hepatitis C virus (HBC) antibodies were all negative.

A month after the latest discharge, our patient returned for a planned visit. His IgE levels were even higher this time, peaking to 15770 IU/ml (normal < 110 IU/ml).

The production of IFNγ was studied as mentioned earlier, in the patient (E), his mother (M) and his father (F). IFNγ was induced in both whole peripheral blood (WPB) and peripheral blood mononuclear cells (PBMC). WPB was diluted 1:10 in RPMI 1640 medium (Gibco, UK) with supplements (see Table). PBMC was separated from peripheral blood on Ficoll–Paque gradient and adjusted to $1 \times \times 10^6$ cells/ml by RPMI 1640 using the same supplements. Induction of IFNγ was performed using mitogens: phytohaemagglutinin (PHA) 5 μg/ml (Serva, Germany) and lipopolysaccharide (LPS) 10 μg/ml, (Sigma, USA), and antigens: HCV core peptides 1–17, 73–91 and NS4 peptide (protease) 1921–1941 and Hepatitis B core antigen (HBcAg), full length core (HBcAg100) protein and with deletions Δ3-12, 105-1-21, 105-1-27, 1 μg/ml. as well as IFNα. The supernatants were harvested for IFNγ detection after 24 h of spontaneous cell cultivation and incubation with PHA and LPS, after 72–96 h of cell incubation with PHA and after 168–192h of cell incubation with antigens. IFNγ in patients cell culture supernatants were measured by ELISA (established at the Institute of Biotechnology, Vilnius, Lithuania) using matched-paired antibodies, products of anti-IFNγ monoclonal antibody (GIFN-10) and peroxidase-labeled anti-IFNγ monoclonal antibody (GIFN-3), [sensitivity 5 IU/ml]. Levels of IFNγ lower than 5 IU/ml were indirectly deduced from the spectrophotometric, optical density measurement (wavelengths *vs*. IFNγ graph) standard values (not presented in this article).

Table. **IFNγ production by monocytes taken from the patient (E), his father (F) and his mother (M) on stimulation with various mitogens. Please refer to text for various abbreviations**

Stimuli	E		M		F	
	WPB	PBMC	WPB	PBMC	WPB	PBMC
Sp (no mitogen)	0	1	0	0	0	1
PHA 24 h	0	5	0	1	0	4
PHA 96 h	1	6	0	6	12	15
LPS	0	1	0	1	0	1
HCVi (1–17)	0	0	1	1	2	1
HCViii (73–19)	0	0	1	1	1	0
NS 4	2	0	1	2	1	2
HBcAg	2	0	2	0	1	2
IFNα	0	0	0	1	0	1
HBcAg 100(full)	1	0	0	0	4	0
HBcAg Δ3–12	1	0	1	0	1	0
Δ105-1-21	1	0	1	0	5	0
Δ105-1-27	1	0	1	0	1	0

RESULTS AND DISCUSSION

The data summarized in Table show the following results:

1. In the PBMC sample the son recorded the lowest response rate in most of the cases (9/13, 69%) in comparison to that of the mother (5/13, 38%) and father (5/13, 38%).

2. The results for the WBP sample indicated that the son and his mother showed a similar number of zero or non-detected responses (6/13, 46%) in comparison to the father's response (4/13, 30%).

3. The highest intensity of response was recorded for the father of our patient, who showed best results at least in response to PHA96, while the response to other stimuli were either slightly higher, similar or lower than that of the mother.

4. The mother and son showed an overall greater number of similarities in their IFNγ response profile.

The relative decrease and sometimes total absence of IFNγ, an important cytokine for chemotaxis and activation of phagocytosis by polymorphonuclear cells (neutrophils, for example), when stimulated by various antigens may in part explain the

frequency of infections in patients with HIES when compared with normal individuals. Our study does seem to suggest that indeed our patient is not an exception.

On the other hand, in many, but not all, of the HIES patients studied so far an autosomal dominant mode of inheritance with variable expressivity seems to be the law. A mutation of a single gene, different genes, or the deletion of adjacent genes have been thought to represent the etiology of HIES (7, 9). In a study were involved 19 kindreds with HIES in whom chromosome number four has been found to contain a disease locus (4q) for HIES (3, 10).

However, regardless of what exact etiology underlies HIES, the persistent elevation of IgE in sera of HIES patients coupled with a normal level of other classes of immunoglobulins is thought to disturb neutrophil and phagocyte chemotaxis thus rendering the patient more susceptible to recurrent infections. The Th2 cells produce several cytokines including interleukin-4, interleukin-5, interleukin-6, interleukin-9 and interleukin-10. Interleukin-4 stimulates the B cells to switch to IgE production, and interleukin-10 suppresses the activity of Th1 cells, the other subset of CD4+ cells. Th2 cytokines also activate eosinophils. Th1 cells can, on the other hand, inhibit the secretion of cytokines by Th2 cells and hence inhibit IgE synthesis as well, via their release of IFNγ. In patients with HIES, a disbalance situation favouring Th2 cells is thought to exist. Another population of T cells, the T suppressor the cells, are also thought to be deficient or less effective in suppressing the overactive Th2 cells in HIES (1, 6).

Recent interest has shifted towards investigating a possible mutation in the alpha subunit of the interleukin-4 receptor (3). Another study found a cytokine profile in one HIES patient of a Th0 type. The majority of the T cells isolated were of CD4+ type, but when investigating the expression of different cytokines by these cells both interleukin-4 and interferon gamma but no interleukin-2 have been detected. The authors suggested that a defective mechanism may lead to accumulation of T cells with Th0 type in HIES (7, 11).

Thus, as mentioned earlier, the defective production of IFNγ, a powerful activator of polymorphonuclear (PMN) cells, is the reason behind defective chemotaxis of PMN cells and increased IgE due to unopposed interleukin-4 effect on B cells. T cells respond to interleukin-12 stimulation abnormally, by significantly lower levels of INF release (8, 12).

CONCLUSIONS

Based on the above results we may suggest that our patient's immune response is similar to what have been found by other investigators and does support, in addition to the clinical and laboratory findings, the diagnosis of HIES syndrome.

Furthermore, though our patient's father has a history of allergic diseases, our results suggest that it is the mother's response profile and not that of the father's that suggests an inheritance pattern, if any, from her side. To solve this dilemma the final word is left to genetic studies.

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