

Short Report

Clinical improvement and normalized Th1 cytokine profile in early and long-term interferon- α treatment in a suspected case of hyper-IgE syndrome

Benninghoff U, Cattaneo F, Aiuti A, Flores-D'Arcais A, Gelmetti C, Viscardi M, Callegaro L, Mirolo M, Ambrosi A, Roncarolo MG, Bacchetta R. Clinical improvement and normalized Th1 cytokine profile in early and long-term interferon- α treatment in a suspected case of hyper-IgE syndrome.

Pediatr Allergy Immunol 2008; 19: 564–569.

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We are reporting on a 7-months-old boy with suspected hyper-IgE syndrome, presenting with a therapy resistant severe eczema and an overall reduction of *in vitro* cytokine production. Interferon- α (IFN- α) treatment resulted in a marked and stable clinical improvement and normalization of *in vitro* T-cell cytokine production, indicating a valid therapeutic potential of IFN- α as immunomodulating drug.

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Key words: interferon- α ; hyper-IgE syndrome; cytokines; immunodeficiency; eczema

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Accepted 8 October 2007

Hyper-immunoglobulin E (IgE) syndrome (HIES) is a rare primary immunodeficiency, characterized by eczema, elevated total IgE, eosinophilia and recurrent infections of skin and respiratory tract. Although sporadic, autosomal-dominant and autosomal-recessive forms of HIES have been commented upon. Together with immunological disorders, defects of the skeleton and connective tissue (such as scoliosis, pathological fractures, failure of dental shedding, hyper-extensibility of joints and characteristic facial phenotype) can be present in the sporadic

and autosomal-dominant forms, becoming worse with age. On the contrary, viral infections and neurological complications are more common in the autosomal-recessive type of HIES. Current therapeutic approaches are mainly symptomatic including prophylaxis and management of bacterial, viral and fungal infections, or immunosuppression with steroids or cyclosporin A.

The aetiology of the disease remains unknown. Dysfunctional T-cell responses with an imbalanced T helper lymphocytes type 1/T helper lymphocytes type 2 (Th1/Th2) cytokine profile

have been suggested to play a role in the pathogenesis of HIES. A predominant Th2 response and a defective Th1 response have been proposed to facilitate immunoglobulin class switching towards IgE.

Based on these considerations concerning the pathogenesis of HIES, it can be hypothesized that the use of an immunomodulating drug, strengthening a Th1 immune response in HIES patients might be a therapeutic option. Interferon- α (IFN- α) has been found to promote the survival and differentiation of antigen-activated Th1 cells. Furthermore, it has been demonstrated that *in vitro* IgE synthesis can be inhibited by IFN- α . Therefore, IFN- α appears to be a potentially useful drug in HIES.

Therapy with IFN- α is currently successfully applied in children suffering from angiomas resistant to steroid treatment and has also been used in some patients affected by severe atopic dermatitis. The therapeutic impact of IFN- α in patients with atopic dermatitis is still debated and does not correlate to an amelioration of serum IgE levels. However, in some atopic dermatitis patients, non-responsive to IFN- γ , a significant improvement of skin lesions has been observed after IFN- α administration.

Based on these observations, we decided to use IFN- α in a 7-month-old patient with suspected HIES, with the aim of modulating the immune response by influencing maturation towards Th1-lymphocytes. The patient's peripheral blood mononucleated cells (PBMC) cytokine production was measured before and during IFN- α therapy.

Methods

Determination of cytokines

Cytokine production by PBMC of the patient was tested approximately every 2 months upon *in vitro* stimulation by monoclonal CD3 antibody (CD3mAb) and monoclonal CD28 antibody (CD28mAb) or by PHA, as delineated.

Controls

Twenty-three age-matched healthy controls were included, under parents' informed consent, in a specific protocol allowing research studies in biological materials from normal donors. At least one control was always assayed at the same time as the patient. The other controls were analyzed in the same way but at different times and provided age-matched normal values as internal standard.

Patient consent/ethical permission

An informed consent for the patient's studies was obtained from both parents under a protocol approved by the Internal Ethical Committee. The consent allows publication as long as the patient's sensitive data are kept secret.

Statistical analysis

Differences between the patient and controls were evaluated with one tail Wilcoxon rank sum test, p-values < 0.05 were considered significant. For controls, median values and Inter Quartile ranges were considered as central values and boundaries.

Case report

The patient presented is the first child of non-consanguineous parents. The family history does not reveal other cases of HIES. The patient was born at term without complications. He suffered from an erythematous, desquamative, exsudative eczema with severe pruritus on his face, trunk and limbs since the age of 1 month; he had perforating otitis media, *bronchitis* and genital candidiasis in his 2nd and 3rd months of life. He was first seen at our centre at the age of 4 months, presenting with a worsening of the eczema described above, superinfected with *Staphylococcus aureus*, increased total lymphocyte counts (18,450/ μ l), eosinophilia (2500/ μ l), elevated IgE levels (3880 U/ml) and hypogammaglobulinaemia [immunoglobulin G (IgG) 62 mg/dl]. Except for the eczema, physical examination did not show any abnormalities. RAST tests resulted strongly positive for 11 food allergens.

As the proportion of the main lymphocyte subsets (CD3, CD4, CD8, CD19, CD16/56) was within the normal range and the *in vitro* proliferative responses to mitogens and *Candida* were comparable to those of age-matched normal donors (Table 1), severe combined immunodeficiency could be excluded. HLA-typing analysis and detection of CD3⁺ CD45RA⁺ peripheral lymphocytes by flow cytometer analysis ruled out erythroderma secondary to maternal engraftment in Omenn syndrome. Hypereosinophilic syndrome and Netherton syndrome were excluded by clinical evaluation, laboratory tests and imaging. Moreover, the unusual hyper-IgE amount and the clinical presentation disqualified a merely Transient Hypogammaglobulinemia of Infancy.

Based on the described clinical manifestations and laboratory findings, the diagnosis of HIES

Table 1. The patient's blood count, lymphocyte subsets, serum immunoglobulin levels, proliferative responses to mitogens and vaccines before and under IFN- α -therapy

	Before IFN- α -therapy	Six months IFN- α -therapy	Twelve months IFN- α -therapy	Twenty-four months IFN- α -therapy
Age (months)	4	13	19	31
WBC ($10^9/l$)	24,600	12,600	14,800	9100
Neutrophils ($10^9/l$)	3200	1890	2700	2100
Lymphocytes ($10^9/l$)	18,450	9300	9700	5900
Lymphocyte subsets				
CD3	7300	6000	5800	3600
CD3CD4	5200	4300	4100	2300
CD3CD8	2100	1700	1700	1300
CD19	4120	2500	1800	1500
CD16/56	850	380	850	310
Eosinophils ($10^9/l$)	2500	760	1360	500
Haemoglobin (g/dl)	13.5	13.0	13.8	13.5
Platelets ($10^9/l$)	760	380	440	390
IgE (U/ml)	3880	3300	7100	1340
IgG (mg/dl)	62 (>222*)	330†	470†	480 (>462*)
IgA (mg/dl)	16 (>6*)	62 (>17*)	165 (>17*)	56 (>27*)
IgM (mg/dl)	25 (>28*)	31 (>48*)	45 (>48*)	50 (>62*)
Response to vaccines	Not evaluable because of incomplete vaccinations	Not evaluable because of IVIG infusion	Not evaluable because of IVIG infusion	Present (TT, HBV)
Responses to mitogens‡,§ (cpm $\times 10^{-3}$)				
Medium	2.5 $\pm$ 0.4	0.8 $\pm$ 0.4	0.25 $\pm$ 0.03	0.54 $\pm$ 0.2
PHA	236 $\pm$ 1	Not done	183.7 $\pm$ 2.6	Not done
α CD3i	48.9 $\pm$ 3.5	61.4 $\pm$ 4	9.3 $\pm$ 0.9	71.7 $\pm$ 3.9
α CD3i + sol α CD28	120 $\pm$ 7.3	211.7 $\pm$ 14.7	43 $\pm$ 1.2	161.8 $\pm$ 10.5
Responses to antigens	Positive to Candida	Not done	Positive to Candida, Tetanus Toxoid and alloantigen	Positive to Candida, Tetanus Toxoid and alloantigen

*Minimum normal value according to age.

†On IVIG therapy.

‡Respective values obtained from healthy controls; Med $\geq$ 0.42; PHA $\geq$ 161.3; α CD3i $\geq$ 61.2; α CD3i + sol α CD28 $\geq$ 134.4;

§More details in Methods.

was strongly considered. According to the Grimbacher score as a reference for the diagnosis of HIES, this patient could be included in the group of patients with 'likely' diagnosis of HIES, considering the absence of some clinical characteristics at an early age. In this regard, the patient did not show manifestations of recurrent pulmonary infections, probably because the treatment prevented their occurrence. However, in the absence of molecular or genetic tests to prove the diagnosis of HIES at the time of initial observation, severe atopic dermatitis could not be completely excluded.

Antibiotic treatment was constantly maintained, at either therapeutic or prophylactic dosage. Because of persistent IgG levels below 400 mg/dl, periodical immunoglobulin infusions have been required. Administration of topic and systemic steroids, topic pimecrolimus 1% and tacrolimus 0.03–1% did not show a stable improvement. Therefore, administration of IFN- α (600.000 U/m² three times a week subcutaneously) was started at the age of 7 months. Clinical examination especially of the skin, full

blood count, immune-phenotype of lymphocytes, determination of serum IgE- and IgG-levels and *in vitro* cytokine production patterns of the patient's T cells have been performed regularly to evaluate the efficacy of the IFN- α -treatment. Monitoring of potential side effects included surveillance of symptoms unrelated to the disease and controls of kidney-, liver-, thyroid-functions and autoantibodies. Apart from a moderate increase of the body temperature (max. 37.7°C) after the administration of IFN- α -injections, this therapy was well tolerated.

Two weeks after the beginning of IFN- α -therapy, a marked improvement of the eczema and pruritus could be noted. During the first year of therapy, the incidence of infections dropped dramatically. Only 9 months after the IFN- α -treatment started, the patient had one severe episode of skin infection as a result of *Staphylococcus aureus*, superinfected with Herpes simplex virus type 1 requiring i.v. antibiotic and antiviral therapy. After this infection, the dose of IFN- α was doubled to 1.200.000 U/m² s.c. three times a week for 3 months but was then tapered

down to the original dose because of an increase in body temperature and vomit in the days of IFN- α -injections, and was maintained at the dosage of 600.000 U/m² s.c. three times a week until now.

Overall, serum IgE levels remained elevated (3.000–10.000 UI/ml, Table 1) without correlation to the stable clinical improvement. However, they were clearly lower when higher dosage of IFN- α was given. IgG production slowly increased and normalized, allowing discontinuation of IVIG replacement (at 2 yr of age) (Table 1). IgM also improved although it remained below normal values (Table 1). Furthermore, the patient showed his own antibody response to vaccine antigens during IFN- α therapy, after discontinuation of IVIG infusion. Cytokine production by PBMC of the patient was tested approximately every 2 months.

At diagnosis, an overall reduction of interleukin-2 (IL-2), IFN- γ , IL-4, IL-5, IL-10 and TNF- α (statistically significant for IFN- γ , $p = 0.041$ and TNF- α , $p = 0.038$, one tail Wilcoxon rank sum test), compared with age-matched normal controls, was detected upon CD3mAb and CD28mAb stimulations (Fig. 1), whereas the amount of cytokines secreted upon PHA-stimulation was normal (not shown). A normalization of the patient's cytokine production (no statistically significant difference between normal controls and patient, all p -values > 0.05) was observed within 4 wk after the beginning of IFN- α -therapy and it remained stable throughout the course of the disease. Overall, a significant increase in Th1-cytokines was detected

under IFN- α -treatment which could be associated with a clear clinical improvement.

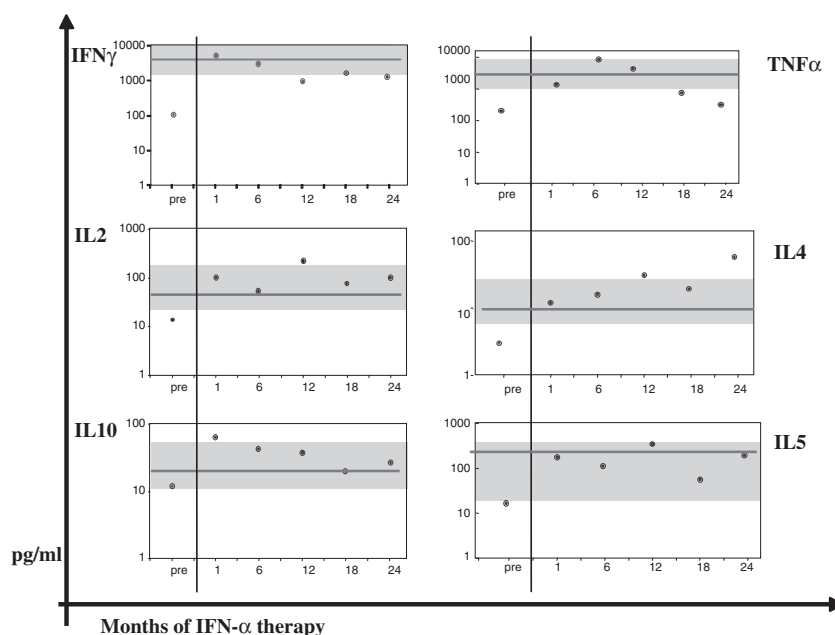
Discussion

Only few HIES patients treated with interferons (IFNs) have been described in literature. Administration of IFN- γ resulted in an improvement of the eczema and a decrease of serum IgE levels in these patients. So far, the use of IFN- α to treat HIES patients has been very limited and restricted to patients affected by advanced HIES. Partial improvement of widespread oral papillomas without the benefit on the recurrent sinopulmonary infections has been reported in a 7-yr-old girl. In a 17-yr-old woman, complete disappearance of the eczema was noted and successful treatment of molluscum contagiosum in a 9-yr-old boy has been described recently.

In our patient, a clear improvement of the eczema and a considerable reduction of infections could be observed. In contrast to the patients reported in literature, our patient was treated with IFN- α at an early stage of the disease and received prolonged low-dose IFN- α treatment (2 yr until now). Furthermore, cytokine profiles, measured under IFN- α therapy, showed a significant increase in Th1-cytokines in parallel with the clinical improvement.

This is the first report of long-term follow-up of cytokine profiles of a HIES patient under IFN- α therapy, which allows us to find a correlation regarding the way of functioning of IFN- α in HIES patients and its underlying pathogenic factors. On the one hand, publica-

Fig. 1. *In vitro* PBMC cytokine production before and under IFN- α -therapy, stimulation: CD3mAb + CD28mAb, detection after 24 (IL-2) or 48 h (other cytokines) by cytometric-bead-array, BD. No cytokines were detected from unstimulated PBMC. Patient's values compared with 23 age-matched controls. Horizontal line: median of controls. Shaded area: Inter Quartile range of controls (1st–3rd quartile).



tions suggest a dysfunctional T-cell response with a predominant Th2 and a defective Th1 pattern, as an important factor in the pathogenesis of HIES. Immunoglobulin class switching towards IgE could be facilitated by this predominant Th2 response. Indeed, it has been demonstrated that *in vitro* IgE synthesis can be inhibited by IFN- α and IFN- γ , whereas it is induced by IL-4. On the contrary, it is known that immunomodulating actions of IFN- α include its ability to promote survival and differentiation of antigen-activated Th1 cells.

In our patient, a significant increase in Th1-cytokines, contemporarily with a clear clinical improvement, was detected under IFN- α -treatment. IFN- α -therapy may have contributed to the improvement of the disease's clinical outcome and possibly to the immune dysregulation, although IgE levels were not decreased. Indeed, a marked improvement could be observed as the overall number and intensity of infections dropped. The reason for elevated IgE levels in our patient remains unexplained. It has been described in literature that in many HIES patients, the extent of clinical symptoms does not correlate with the amount of serum IgE. It could be speculated that in our patient the dosage of IFN- α used was sufficient for an immune modulating effect, leading to a clinical improvement, but too low to influence IgE production, or that T-cell-independent mechanisms of IgE regulation might be involved.

After initial increase in months 6 and 12, a reduction of TNF- α production back to the pre-treatment levels could be observed in months 18 and 24 (Fig. 1). Considering lower TNF- α production as a sign of lower inflammatory activity, this finding correlates with the child's much better clinical conditions in months 18 and 24.

Our patient received prolonged IFN- α treatment. Nevertheless, he did not show any severe side effects. This is an important observation as it has been shown that IFN- α has transient effects, and therefore long-term treatment seems to be necessary as indeed is the case in the patient we report.

Possible side effects associated with long-term IFN- α therapy, including vomiting, diarrhoea,

psychological disorders, neutropenia, anaemia, thrombocytopenia, constipation, somnolence and sleep disorders, development of autoimmunity such as Systemic Lupus Erythematosus, were not experienced in our patient.

It has been stated that instead of using the term hyper-IgE syndrome, the term hyper-IgE syndromes should be used more correctly to indicate the existence of a group of diseases, characterized by clinically similar features but most likely caused by different molecular defects and possibly requiring different therapeutic approaches. For instance, IFN- α could be an effective therapy for some but not for all HIES patients: IFN- α does not seem to be of any benefit for HIES patients affected by a homozygous mutation of tyrosine kinase 2, causing an impairment of signalling pathways of several cytokines including type I IFN. However, the association of tyrosine kinase 2 mutations with AR-HIES seems to be a rare condition.

Further studies with an extended cohort of patients and longer follow-up will be necessary to evaluate the therapeutic benefit of IFN- α and its potential role in modulating the cytokine patterns in HIES patients, which could provide more insights into pathogenic aspects of this disease involving dysfunctional T cells with altered cytokine patterns, once the genetic defects will be dissected.

It remains to be clarified, whether this therapy could also have beneficial effects on the function of other cell types, such as endothelial cells, dendritic cells, monocytes or macrophages, whose defects could be involved in the pathogenesis of this multiorgan disease.

The current good health of the child with normal thriving and normal psychomotor development despite numerous food allergies prompts us to speculate that IFN- α -therapy in HIES could have a broader beneficial effect beyond that of modulating cytokine production pattern.

Conclusion

Long-term IFN- α treatment started at an early stage of the disease may be of considerable therapeutic benefit for some HIES patients, unresponsive to conventional immunosuppression.