



Effect of Recombinant IFN- γ on IgE-Dependent Leukotriene Generation by Peripheral Blood Leukocytes in Patients with Pollinosis and Asthma

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Abstract. Interferon γ (IFN- γ) is considered one of the causative and intensifying factors in inflammation. The reaction to allergens releases IFN- γ , an immunomodulatory cytokine known to inhibit IgE synthesis and Th cell proliferation. The aim of the study was to evaluate the influence of IFN- γ on leukotriene (LT) release *in vitro*, from human leukocytes of atopic patients with pollinosis and asthma. Thirty-eight patients were enrolled in the study: 15 with pollinosis and 23 asthmatics. In the presence of IL-3, leukocytes were stimulated with specific allergens. Other samples of leukocytes were preincubated with different concentrations of IFN- γ for 15 min before allergen stimulation. The concentration of LT in supernatants was measured according to the CAST-ELISA procedure. We stated that IFN- γ had significantly diminished LT release in a dose-dependent mode from the leukocytes of pollinotics. IFN- γ did not change LT release in the asthmatic group, although, in leukocytes the small and medium basic production of LT, IFN- γ caused a statistically significant fall in LT generation.

Key words: asthma; pollinosis; interferon γ ; leukotriene.

Introduction

The relationship between viral respiratory tract infections and bronchial asthma exacerbation is commonly known¹. Viral infections are also responsible for the increase in bronchial hyperactivity, which is not always transient. The pathomechanism of this phenomenon has not been fully explained so far, although many of its elements are known. Viruses cause reversible damage of the bronchial epithelium which makes it permeable to inhaled irritant substances and allergens. They also stimulate inflammatory mediator liberation from macrophages, mast cells and eosinophils and viral infection is associated with a rise in free oxygen radical production^{1, 5, 7, 8}.

Interferon (IFN) induced by viruses is considered one of the causative and intensifying factors in inflammation. In our previous work we observed bronchospastic reactions in asthmatics after leukocyte derived (type I) IFN inhalation¹⁷. Some authors⁶ found an elevated release of IFN by bronchoalveolar leukocytes from patients with asthma. The reaction to allergens causes the release of IFN- γ (type II) from T and NK lymphocytes. This is an immunomodulatory cytokine known for inhibiting IgE synthesis and Th-cell proliferation. CD8⁺ lymphocytes are considered as potential producers of IFN- γ ¹⁵. This is consistent with the finding of a significant inverse correlation between the proliferative response and the ratio of IL-4/IFN- γ production²⁰. Although IFN- γ has biological functions com-

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mon with other IFNs, its antiviral activity is several times lower, whereas its immunomodulating action is much more expressed compared to that of IFN type I. It is synthesized by Th1 lymphocyte clones together with interleukin 2 (IL-2), lymphotoxin and tumor necrosis factor (TNF). These cytokines play important roles in inducing T lymphocyte and macrophage effector functions⁴. In general, IFN- γ is not thought to be an inflammatory mediator, but, in the presence of TNF, it can synergistically enhance the inflammatory function of the latter⁶. IFN- γ and TNF can stimulate macrophages, neutrophils or endothelial cells to release reactive oxygen or nitrogen intermediates, essential in the destruction of pathogens and tumor cells. Incubation of macrophages with only IFN- γ triggers the production of both oxygen and nitrogen reactive products (H_2O_2 , NO_2). From the allergologist's point of view, most important is the fact that IL-4 and IFN- γ display counter-regulatory effects on IgE production²⁵. This was the basis of several studies in which IFN- γ was used *in vitro* as well as *in vivo*^{11, 13}. Establishment of the influence of IFN- γ and other cytokines on an effector cell's reactivity stimulated by a specific allergen seems to be very important, as it can result in a better knowledge concerning the intensity of allergic symptoms mediated by a specific allergen challenge *in vivo*. The present study was conducted in order to evaluate the influence of IFN- γ on *in vitro* allergen induced leukotriene (LT) release from human leukocytes obtained from atopic patients with pollinosis and asthma.

Materials and Methods

Thirty-eight patients were enrolled in the study, consisting of 15 patients, 9 males and 6 females aged 16 to 38 years (mean 24.9) with pollinosis and 23 patients, 8 males and 15 females aged 19 to 47 years (mean 38), with mild and moderate atopic asthma. The pollen-allergic patients were in remission stadium and the asthmatics received only β -mimetics on demand or topical steroids. Diagnosis was based on anamnesis, physical examination and positive results of the skin prick tests as well as spirometric evaluation. The asthmatics fulfilled the criteria for diagnosis of asthma as defined by GINA¹⁰.

The Ethics Committee of the University Medical School, Wrocław, approved the study and the patients gave informed written consent.

Generation of leukotrienes was measured following the CAST-ELISA procedure. It was performed using Bühlmann Laboratories A. G. kits in accordance with

the manufacturer's instructions. According to KURIMOTO et al.¹⁹, more than 95% of all biological activity of blood leukotrienes depends on basophil activation expressed as LTC_4 release. Peripheral blood (5 ml) was drawn on the EDTA and mixed with dextran solution. After 90 min, the upper phase was transferred to the next polypropylene tube and centrifuged. The platelet-rich supernatant was decanted and the cell pellet was suspended in a stimulation buffer containing IL-3. The cell suspension was aliquoted to the separate tubes and allergen portions (*Dermatophagoides pteronyssinus* or grass pollen mixture-Bühlmann Laboratories A. G.) were added to a final concentration of 20 ng/ml. In the other tubes, leukocytes were preincubated for 15 min with IFN- γ (Sigma) in concentrations of 10, 100 and 1000 UI/ml and then stimulated with the mentioned allergens. In addition, each patient's cells were tested for spontaneous sulfidoleukotriene production (negative control) and with anti-IgE antibody (as a positive control). The cells were incubated for 40 min at 37°C. Finally, the cells were centrifuged and the cell supernatants were frozen for a period of 14 days and then tested for levels of sulfidoleukotrienes. The ELISA procedure was performed using precoated microtiter plates. Enzyme conjugate and antibody solution were added to the cell supernatants as well as to the standards and incubated for 18 h at 4°C. After the washing step, freshly prepared substrate solution (p-nitrophenylphosphate) was added to each well and incubated for another 30 min at ambient temperature. Finally, stop solution (1 M NaOH) was added to each well and color absorbancy was measured at a wavelength of 405 nm in a microtiter plate reader (Stat-Fax 2100, Awareness Company USA). Leukotriene production was expressed in pg/ml after subtraction of values of spontaneous leukotriene production. The results of the allergen stimulation were regarded as positive if the net sulfidoleukotriene concentration was 200 pg or more, relative to spontaneous leukotrienes secretion.

This procedure allows measuring C4, D4 and E4 leukotriene concentration together due to the affinity of the applied monoclonal antibodies in the ELISA step.

The results were statistically analyzed by Student's *t*-test. All analyses are expressed as mean $\pm$ SD. A *p* value of less than 0.05 was considered significant.

Results

Spontaneous leukotrienes generation ranged from 0 to 78 pg/ml (mean 63.2 pg/ml, SD = 21.3). After anti-IgE antibody stimulation mean leukotrienes gener-

Table 1. Influence of IFN- γ on leukotriene formation by PBLs of pollinotics (n=15)

	Leukotriene concentration (pg/ml)			
	stimulation with allergen	incubation with IFN- γ		
		10 UI/ml	100 UI/ml	1000 UI/ml
X	1320.9	1043.5	842.76	473.07
SD	799.75	590.0	498.8	372.32
p		ns	ns	0.006

ns – not significant.

Table 2. Influence of IFN- γ on leukotriene formation by PBLs of asthmatics (n=23)

	Leukotriene concentration (pg/ml)			
	stimulation with allergen	incubation with IFN- γ		
		10 UI/ml	100 UI/ml	1000 UI/ml
X	1796.6	1792.3	1906.3	1784.0
SD	1140.1	1306.2	1425.8	1374.4
p	ns	ns	ns	ns

ns – not significant.

Table 3. Influence of IFN- γ on leukotriene formation in subjects with low basic release of LTs (n=8)

	Leukotriene concentration (pg/ml)			
	stimulation with allergen	incubation with IFN- γ		
		10 UI/ml	100 UI/ml	1000 UI/ml
X	628.3	376.9	404.1	322.5
SD	276.8	379.0	297.2	233.3
p		ns	ns	0.042

ns – not significant.

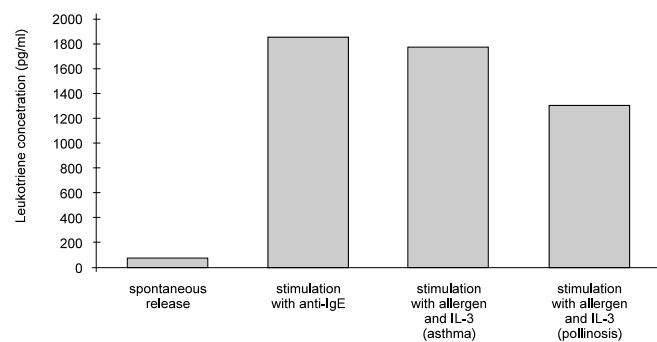
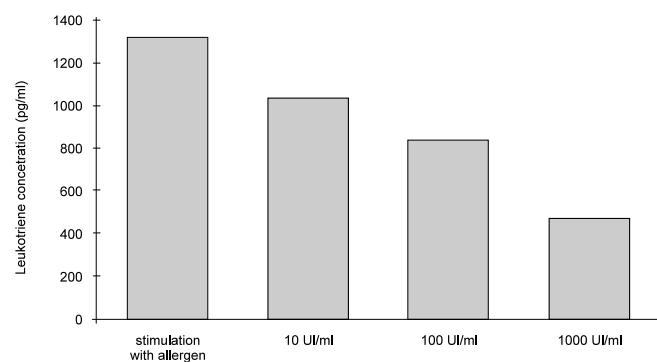
Table 4. Influence of IFN- γ on leukotriene formation in subjects with high basic release of LTs (n=15)

	Leukotriene concentration (pg/ml)			
	stimulation with allergen	incubation with IFN- γ		
		10 UI/ml	100 UI/ml	1000 UI/ml
X	2419.6	2547.2	2707.5	2563.5
SD	914.5	949.4	1106.6	1058.5
p		ns	ns	ns

ns – not significant.

ation increased to 1854.3 and ranged from 1123 to 2564.2 pg/ml (SD = 768.4 Fig. 1).

The mean allergen dependent leukotriene generation observed in pollinotics was 1320 pg/ml, SD = 799. IFN- γ in all applied concentrations caused a fall in leukotriene generation of the pollinotics' leukocytes when compared to the control (Table 1, Fig. 2). In concentrations of 10 and 100 UI/ml, leukotriene concentrations were 1043.5 and 842.76 pg/ml, respectively. But only

**Fig. 1.** Spontaneous, anti-IgE and allergen – induced leukotriene release from peripheral blood leukocytes in subjects with asthma and pollinosis**Fig. 2.** Influence of IFN- γ on leukotriene release from peripheral blood leukocytes stimulated with allergens in subjects with pollinosis

at an IFN- γ concentration of 1000 UI/ml was the fall statically significant ($p=0.006$), and the mean LT concentration was 473.07 pg/ml.

Leukocytes obtained from asthmatic patients released a mean of 1797 pg/ml LT in the presence of IL-3. When incubated with IFN- γ , the release of LT did not change and it was not dependent on interferon concentration (Table 2).

Among the 23 asthmatics, leukocytes from 8 patients basically (after allergenic stimulation) released small and medium amounts of LTs (mean 628.3 pg/ml). After incubation with 1000 UI/ml of IFN- γ we found a significantly reduced production of LTs (Table 3). In the remaining 15 persons we noticed a high generation of LTs (mean 2420 pg/ml) and in that case incubation of leukocytes with IFN- γ did not change LT release (Table 4).

Discussion

IFN- γ is an immunomodulatory cytokine which inhibits synthesis of IgE as well as the proliferation and

activity of Th2 lymphocytes^{5, 12}. Th0 lymphocytes of people with an atopic defect recognise antigen peptides presented by class II major histocompatibility complex (MHC) molecules on antigen presenting cells (APC) through antigen specific receptors. This complicated process is the signal for Th0 cells to differentiate into the Th2 lymphocyte²⁵. These lymphocytes produce numerous cytokines (IL-3, IL-4, IL-5, IL-9, IL-10, IL-13, GM-CSF and others), taking part in induction of IgE synthesis by B lymphocytes (IL-3 and IL-4) and inhibiting Th1 lymphocytes related to the competitive immunologic response^{18, 29}. The characteristic feature of the Th1 lymphocyte subpopulation is the release of IFN- γ and TGF- β , both cytokines that activate macrophages and support the formation of granulation. The existence of T lymphocyte subpopulations and the meaning of their role in the inflammatory process¹³ was the background of experimental investigations involving clinical trials. These were based upon the issue that IFN- γ and IL-4 demonstrate opposite regulatory activities^{6, 21}. The results of those investigations are not equivocal. IFN- γ used in trials did not fulfil previous hopes, although the findings of BOGUNIEWICZ et al.³ concerning bronchial asthma should be mentioned. The beneficial effect of IFN treatment in atopic skin inflammation was only transitory¹¹. *In vitro* experiments performed by our team found an neither influence of IFN on the chemiluminescence of the mononuclear blood cells of people with bronchial asthma nor an influence of IFN on the production of the eosinophil activating factor^{14, 16}. The study of IFN-induced modification of mediator release from leukocytes seemed to be interesting and it was suggested that a similar effect would be obtained in the case of asthmatic patients as in pollinotics. However, despite the same procedure, IFN- γ did not inhibit the IgE-dependent release of LTs from the immunized basophils of asthmatics (most blood leukocyte LTs are known to be released by basophils). It seems that the cause of this phenomenon is multifactorial. IFN- γ is a lymphokine involved in numerous immunologic and inflammatory processes, cellular recruitment and activation via increased expression of adhesion molecules. In these processes, IFN cooperates with other cytokines. The antiallergic activity of IFN probably depends on cooperation with IL-10. In subjects with allergic manifestations after viral infections, diminished production of IL-10⁹ was observed. The most conceivable evidence of the role of IFN and IL-10 comes from experiments of PIERKES et al.²⁴. They found that IFN- γ and IL-10 are responsible for the decreased release of leukotrienes by human peripheral blood leukocytes (PBL) in patients who are allergic to wasp

venom. In this study, allergen-induced basophil histamine release was also lowered in the presence of these cytokines. The investigation of whether these T cell derived cytokines can influence the histamine and sulfidoleukotriene release from effector cells revealed that addition of IL-10 and IFN- γ to PBLs before wasp venom challenge led to a dose-dependent diminishing of venom-induced histamine and LT release. Our results are in line with that study, for we have demonstrated in pollinotics a dose-dependent decrease of LT release from allergen-stimulated PBLs after preincubation with IFN- γ . These findings provide evidence for the influence of IFN on the releasability of effector cells. This is not surprising, because it is known that cytokines can modulate the effector phase of IgE-mediated reactions¹⁹. The suppressive effect of IFN- γ on the effector phase of the IgE mediated reaction can also explain the early benefits of specific immunotherapy, where reduced target cell reactivity cannot be explained by a depression of IgE synthesis, because the level of this immunoglobulin still remains high.

We cannot explain why a dichotomy in the reactions to IFN in the PBLs of the asthmatic group exists. In our study we used PBLs of people with atopic diseases: asthma or pollinosis. All investigated subjects were immunized to grass pollen or mites. Those allergens were used to induce the LT release. From this point of view, the material seemed to be homogenous. But from the clinical point of view (different stages of severity, various pharmacotherapy in asthma patients, untreated pollinotics in remission) and considering the pathogenesis of the disease, the asthmatic patients were not an homogenous group. The model of basophil response to IFN- γ demonstrates the special importance. We discovered that cells which were stimulated with an allergen and IL-3 and released much more LT did not respond to incubation with IFN- γ . However, we noted that 8 persons demonstrated a significant dose-dependent decrease of LT release under the influence of IFN- γ . The basophils of these patients initially released smaller amount of leukotrienes. The reason for this phenomenon is unclear; it is possible that, in the cases with very high response to allergen stimulation, the concentration of IFN- γ is too low to express sufficient suppression of LT generation.

The observed dichotomy of the basophil response to IFN might be incidental, or it might result from some unknown process caused by qualitatively and quantitatively different states of the induced cytokines. IFNs cooperate with other cytokines and the result of that cooperation may depend on numerous factors, mostly unknown. There are very few investigations on IFN- γ

-influenced induction and LT release reactions. NAS-SAR'S et al.²² experiments revealed that the opposing activity of proinflammatory cytokines originating from the Th2 subpopulation goes along different paths. The authors examined the influence of IL-1 and IFN- γ (cytokines of Th1 lymphocytes) as well as IL-4 and IL-13 (cytokines of Th2 lymphocytes) on the production of LTB₄. They showed that IL-1 and IFN stimulated LT release from monocytes activated with ionophor while IL-4 and IL-13 inhibited that release. The same authors stated that IFN- γ inhibited 15-lipoxygenase activation, induced by IL-13²³. IL-4 acts similarly to IL-13. This study proved that cytokines originating from Th1 lymphocytes have contraregulatory effects to Th2 cytokines. VIRCHOW et al.²⁷ stated that the BALF of patients with nonatopic asthma contains much more IFN- γ , than the BALF of atopic asthmatics. This seems to be another argument for the varying activity of IFN in allergic inflammation. Blaser states that in allergy to insect venom, the IL-4/IFN- γ release ratio depends on antigen concentration, resulting in the production of different immunoglobulines (IgE, IgG4)² which in turn leads to manifestation of allergy or another immunologic reaction.

Quantities of IFN- γ that suppress IgE formation and limit eosinophil migration are generally reduced in asthmatic airways²⁸. Less is known about the role of this cytokine in virus-induced wheezing. Recently, VAN SCHAİK et al.²⁶ found that ratios of IFN- γ to IL-4 were higher (due to increased amounts of IFN) in subjects with wheezing and it correlated with increased concentrations of LTs in respiratory secretions.

The role of IFN- γ in immunological mechanisms including allergy is well confirmed, however many factors need to be elucidated. The bivalent influence of IFN- γ from sensitised basophils on leukotriene generation demonstrated in our study is an important finding, although it is also a basis for further questions.

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