

Immunoglobulin E-Dependent Regulation of the CCR3 Chemokine Receptor by Interferon-Gamma in Atopic Asthmatics

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Key Words

Atopic asthma • Chemokine receptors • Interferon- γ • CCR3 mRNA expression • Peripheral blood leukocytes

Abstract

Background: The chemokine receptor CCR3 mediates the migration of cells that play an important role in the pathogenesis of asthma to inflammatory foci. Interferon (IFN)- γ is known to downregulate the expression of some chemokine receptors. Therefore, we decided to analyze the regulation of CCR3 by IFN- γ in asthmatics and to characterize the dependence of this process on immunoglobulin E (IgE) levels.

Methods: Atopic asthmatics were treated with IFN- γ or placebo, and the IgE concentration in the blood was measured using an ultra-micro-ELISA for total IgE. Mononuclear cells from patients and controls were isolated by Ficoll-Hypaque gradient and incubated in the absence or presence of IFN- γ for different periods of time. After incubation, the cells were washed and lysed for RT-PCR analysis, which was performed using a Perkin-Elmer kit. **Results:** IFN- γ treatment apparently improved the evaluated clinical variables; however, the differences were not significant compared to the placebo group. We found that IFN- γ downregulated CCR3 mRNA expression ex vivo and in vivo in those patients with IgE levels

higher than 500 IU/ml, whereas IFN- γ upregulated CCR3 mRNA expression in patients with IgE levels lower than 500 IU/ml. Correspondence between ex vivo and in vivo results was observed using this approach. There was found to be a direct correlation between total serum IgE and CCR3 mRNA expression. **Conclusions:** In those asthmatic patients with high levels of IgE, who are thus susceptible to downregulation of CCR3 by IFN- γ , a significant therapeutic effect with systemic IFN- γ might be expected.

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Introduction

Asthma evokes chronic inflammatory reactions characterized by a T helper type 2 (Th2)-dominated immune response to allergens mediated by Th2-type cytokines [1, 2] such as interleukins (ILs; e.g. IL-4, IL-5 and IL-13), which results in tissue damage by infiltrating eosinophils, basophils, mast cells and Th2-like lymphocytes [3]. The consequent alteration in the cytokine milieu, with excess Th2 products and decreased T helper type 1 (Th1) products [e.g. interferon (IFN)- γ and IL-12] is predicted to drive the asthma phenotype. Although inhibition of the IL-4/IL-13 system can efficiently prevent the develop-

ment of the allergic phenotype, these cytokines seem to play only a minor role in established allergy [4].

In mice, IL-4, IL-5, IL-9 and IL-13 promote Th2 cell differentiation and B cell-dependent immunoglobulin E (IgE) production, tissue eosinophilia, goblet cell hyperplasia and airway hyperreactivity [5, 6]. It has been difficult to prove a precise role for IgE in the pathogenesis of asthma using murine models of the disease [7, 8], although the findings of these reports do not exclude an important role for IgE in atopy. It is possible that IgE plays a greater role in inhaled allergen-induced bronchospasm and late-phase responses in the airways [9]. Furthermore, these responses are downregulated by Th1 cytokines such as IFN- γ and IL-12 and are inversely correlated with the level of Th1 cell responses [10, 11].

Chemokines are commonly produced and secreted during the immediate hypersensitivity response. They induce circulating leukocyte subsets, such as eosinophils, basophils and Th2 lymphocytes and to a lesser extent monocytes and Th1 cells, to leave the blood stream and migrate to inflammatory foci by a process of chemotaxis [12]. All chemokines signal through G-protein-coupled transmembrane receptors [13]. Th1 cells preferentially express the chemokine receptors CCR5 and CXCR3, whereas Th2 cells predominantly express CCR3, CCR4 and CCR8 [14]. As reviewed elsewhere [15], chemokines contribute to the allergic response not only by directing leukocyte trafficking and degranulation, but also by amplifying the Th2-type immune response of the host.

From several previous studies [16, 17] it is clear that adequate regulation of chemokines and their receptors might benefit the host response by reinforcing Th1-dominated cytokine responses to environmental allergens. In this context, IFN- γ is a good candidate to drive this action.

With regard to its regulation of chemokine receptors, it has been demonstrated that IFN- γ upregulates CCR1 and CCR3 expression in neutrophils [18], CCR5 in macrophages, Th1 and CD34 cells [19, 20] and CCR6 [21] and CXCR4 in eosinophils [22]. Conversely, IFN- γ downregulates CCR2 and CXCR4 in monocytes [23, 24] and neutrophils [25], CCR5 in T cell clones from IL-12 receptor- β 1-deficient patients and healthy individuals [26] and CCR6 in Langerhans cells [27].

CCR3+ eosinophils play an important role in chronic allergic airway disease [28]. Because of its central role in eosinophil recruitment and its expression on Th2 cells, the CCR3 chemokine receptor might be a good target to interfere with the development of asthma. Recently, CCR3 antagonism has been related to inhibition of eo-

sinophil influx into the lung and diminution of asthma pathology [29, 30]. In this work, we analyzed several clinical variables in patients with moderate persistent asthma, as well as the *in vivo* regulation of CCR3 by IFN- γ and its dependence on IgE levels in these patients.

Patients and Methods

Study Design

The study population ($n = 189$) consisted of all Cubans with pathological antecedents of atopic bronchial asthma diagnosed at any time of their life who presented for an allergy consult at the 'Manuel Ascunce Domenech' Hospital in Camagüey, Cuba. Only 50 of them were recruited for the clinical trial.

The patients were randomized in a double-blind study to receive placebo or 5×10^5 IU/ml IFN- γ (Heberon Gamma R) intramuscularly on the same treatment schedule (5 times a week for 4 weeks, then 3 times a week for 4 weeks and finally 2 times a week for 16 weeks). The patients were monitored every 2 weeks for safety and efficacy. All individuals gave their informed consent to participate in the study. The ethical committee of the Manuel Ascunce Domenech Hospital approved the study. The clinical trial was performed in accordance with good clinical practice and the Declaration of Helsinki (48th General Assembly, Somerset West, Republic of South Africa, October 1996).

Study Population

Patients were required to fulfill the following inclusion criteria: male or female outpatients aged between 18 and 65 years (inclusive), with moderate persistent asthma for a minimum of 6 months according to the clinical-functional classification of the Global Initiative for Asthma, which was the result of a consensus between the World Health Organization and the US National Institutes of Health [31]. The recruited patients were from the Manuel Ascunce Domenech Hospital. The subjects had moderate allergic asthma, classified as 'moderate persistent' on the basis of a baseline value for mean forced expiratory volume in 1 s that was between 60 and 80% of the predicted value, reversible with the use of a β -agonist bronchodilator (improvement of 15% after bronchodilator administration) and with variability of peak expiratory flow of more than 30%. None of the patients had received oral steroids in the 8 weeks preceding blood collection. As controls for the *ex vivo* kinetic experiments, age-matched, apparently healthy persons without any symptoms or history of atopy were selected.

Clinical Assessment

The clinical evaluation of patients was carried out using the primary efficacy variables described below:

(1) number of crises: this measures the number of exacerbations of the illness experienced by the patients during the treatment period;

(2) crisis intensity: this is the magnitude of the intensity of the symptoms during the exacerbation, taking into account the specific therapeutic administered, and

(3) symptom persistence: this measures the disappearance or not of the symptoms from the moment in which the evaluated drug or placebo was administered until the final evaluation.

The efficacy of the treatment was measured by the reduction in crisis number, crisis intensity and symptom persistence. The reduction in crisis number was scored as follows: high = reduction in crisis number of more than 70%; moderate = reduction in crisis number of between 40 and 69%; low = reduction in crisis number of between 10 and 39%, and none = reduction in crisis number of less than 10%. The percentage reduction in the number of crises was evaluated taking into account the total number of crises in the year preceding the clinical trial for each patient (taken as 100%).

The crisis intensity was scored as follows: 0 = absence of symptoms; 1 = light symptoms, i.e. resolution of the crisis with inhaled agonist and/or oral theophylline; 2 = moderate symptoms, i.e. resolution of the crisis required, in addition to the above treatment, intravenous theophylline and/or steroid administered by any route, and 3 = serious symptoms, i.e. the crisis was not resolved with any of the previous measures, and hospitalization in an emergency unit for 24 h or more was required.

IgE Determination

Blood samples from asthmatic patients and apparently healthy individuals (controls) were drawn into polypropylene syringes without any anticoagulant. Sera were separated by centrifugation at 1,200 rpm for 5 min at room temperature, aliquoted and kept at -20°C until assay. The time from blood collection until the serum was frozen was between 2 and 3 h. Sera from control individuals were used as negative controls. The IgE level was determined using a sandwich ultra-micro-ELISA for IgE (Tecno-Suma, Havana, Cuba).

Isolation and Incubation of Peripheral Blood Mononuclear Cells

During the clinical trial, we also evaluated the ex vivo regulatory effect of IFN- γ on CCR3 expression by mononuclear (MN) cells. Peripheral blood MN cells from some asthmatic patients recruited during the study were isolated from heparinized blood by Ficoll-Hypaque plus (Sigma) density gradient centrifugation, and the MN cells were collected from the ring. The cells were washed twice with PBS (pH = 7.4) and then resuspended in RPMI-1640 (pyrogen free, Gibco) and 10% fetal calf serum. Then, 3×10^6 cells/ml were incubated in 50-ml Corning tubes with or without 500 or 1,000 IU/ml IFN- γ (Heberon Gamma R) for 24 h at 37°C in a humidified atmosphere of 5% CO_2 .

MN cells from asthmatic patients from a previously conducted small pilot study and healthy volunteers were used to identify the best time for measurement of the ex vivo regulatory effect of IFN- γ on CCR3 expression. Several blood samples from these patients or volunteers were processed and evaluated. Cells were incubated as described above for 2, 24 and 48 h.

Detection of Chemokine mRNA Expression by PCR

mRNA expression of the genes of interest was studied in MN cells using the GeneAmp RNA PCR kit (Perkin-Elmer Cetus, Norwalk, Conn., USA).

The following primer pairs specific for the different genes were used: for CCR3, 5'-TACAGTATATAGCTGGAGGC-3' and 5'-AATGGGATGTATCTGCCAG-3', and for glyceraldehyde-3-phosphate dehydrogenase (GAPDH), 5'-CCATGGAGAAG-GCTGGGG-3' and 5'-CAAA GTTGTCATGGATGACC-3'.

In brief, first-strand DNA was synthesized in a final volume of 20 μl from 1 μg of total RNA in diethylpyrocarbonate- H_2O using the mixture suggested by the suppliers. The mixture was incubated in a DNA Thermal Cycler (MJ Research Inc.) at 25°C for 10 min, then for 1 h at 42°C followed by 5 min at 99°C and then frozen until use (max. 24 h). PCR was performed using the Gene Amp PCR system (Perkin-Elmer Cetus). Complementary DNA (10 μl) was mixed as recommended with the appropriate primers. Amplification was started with 5 min of denaturation at 94°C , followed by 35 PCR cycles. Each cycle consisted of 60 s at 94°C for denaturation, 40 s for annealing (variable temperature depending on primers used) and 40 s at 72°C for extension. The final extension lasted for 5 min at 72°C . The experimental conditions and number of PCR cycles were predetermined to ensure that the amount of cytokine and housekeeping gene fragments were in a linear range of amplification. For that purpose, 5–7 identical PCR reactions were carried out with experimental primers (CCR3 and housekeeping gene) and stopped 1 reaction after every 2 cycles. Amplification products were run on an agarose gel and quantified by densitometry scanning, and the results were plotted on a graph (absorbance vs. cycle number). Total RNA was used as an internal control. The housekeeping gene used was GAPDH and was amplified in 30 cycles.

Semiquantification of PCR Products

Three microliters of loading dye mix was added to the PCR mixture (20 μl) and electrophoresed in an 80-volt constant-voltage field in 2% agarose (Sigma Chemical Co., St. Louis, Mo., USA) containing ethidium bromide. After electrophoresis, the gel was analyzed using the Molecular Analyst software for Windows (version 1.4.1). The results were expressed as a ratio calculated from the OD of a defined area of the amplified chemokine receptor gene products divided by the corresponding OD of the amplified GAPDH PCR product.

Statistical Analysis

Statistical analysis was performed with the SPSS software program for Windows, version 8.0, and Win Bugs, version 1.2. The analysis was nonparametric due to the small size of the samples and because the analyzed data did not follow a normal distribution as verified by the Shapiro-Wilk test. The Mann-Whitney U test was used for the statistical analysis of comparisons between groups. The Wilcoxon rank-sum test was employed for analysis of the IgE values.

Results

Clinical Assessment

Fifty patients were enrolled in the study (females/males: 43/7; mean age: 39.68 ± 11.40 years). The demographic features and disease characteristics of the patient groups are given in table 1. There were no differences between the groups with regard to sex, race, age distribution, initial eosinophil counts or total IgE.

Due to lack of efficacy, the trial was stopped before all patients completed the treatment. Three patients inter-

rupted treatment. Additionally, 7 patients withdrew voluntarily (3 in the placebo group and 4 in the IFN- γ group), and 1 patient from the IFN- γ group withdrew due to worsening of the condition.

The results of the efficacy evaluation are shown in table 2. With regard to the occurrence of crises (crisis number), no significant differences were observed between the treatment groups. In the analysis of crisis intensity, it was observed that patients treated with IFN- γ had no crises of serious intensity, while such crises occurred in 3 patients in the placebo group (7%). The global evaluation failed to show any significant difference between the groups; nevertheless, we found a greater global response in the IFN- γ group (63.2%). With regard to symptom persistence, 84.2% of patients from the IFN- γ group were found inside the categories improved and partly improved, compared to only 70% in the placebo group.

We found the following results for the absolute eosinophil counts. In the placebo group, this parameter tended to increase during the trial [median $\pm$ quartile range (QR) initial absolute eosinophil count = $177.00 \pm 433 \times 10^9/l$, median $\pm$ QR final eosinophil count = $300.00 \pm 493 \times 10^9/l$; Wilcoxon test = 0.207]. Conversely, this parameter was stable in patients from the IFN- γ group throughout the trial (median $\pm$ QR initial eosinophil count = $200.00 \pm 364 \times 10^9/l$, median $\pm$ QR final eosinophil count = $200.0 \pm 163 \times 10^9/l$; Wilcoxon test = 0.408).

IgE Evaluation

The IgE levels from patients who randomly received IFN- γ or placebo were determined before treatment and 6 months after the beginning of the trial. The median IgE concentration showed a tendency to increase in the placebo group during treatment (median $\pm$ QR initial IgE = $253.800 \pm 1,007$ IU/ml, median $\pm$ QR final IgE = 481.338 ± 519 IU/ml; Wilcoxon test = 0.807). However, in the IFN- γ -treated group, the median IgE value dropped twofold (median $\pm$ QR initial IgE = 362.6 ± 745 IU/ml, median $\pm$ QR final IgE = 191.1 ± 620 IU/ml; Wilcoxon test = 0.215).

Differential Regulation of CCR3 mRNA Expression by IFN- γ

The results in table 3 show that after 2 h of incubation, IFN- γ had a tendency to downregulate CCR3 mRNA expression, as has been demonstrated in neutrophils by others [18]. IFN- γ had a significant downregulatory action on CCR3 mRNA expression after 24 h of incubation with 500 IU/ml, an effect that persisted until 48 h. However, in

Table 1. Demographic data of patients enrolled in the clinical trial

	Placebo (n = 25)	IFN- γ (n = 25)
Sex		
Males	5 (20.0)	2 (8.0)
Females	20 (80.0)	23 (92.0)
Race		
White	20 (80.0)	19 (76.0)
Black	3 (12.0)	4 (16.0)
Mestizo	2 (8.0)	2 (8.0)
Smoking		
Nonsmoker	18 (72.0)	17 (68.0)
Ex-smoker	5 (20.0)	7 (28.0)
Smoker	2 (8.0)	1 (4.0)
Eosinophil count, $10^9/l$		
Mean $\pm$ SD	269.20 $\pm$ 221.64	342.36 $\pm$ 368.85
Median $\pm$ QR	164.00 $\pm$ 323.00	226.00 $\pm$ 297.00
IgE, IU/ml		
Mean $\pm$ SD	626.99 $\pm$ 814.80	435.22 $\pm$ 531.22
Median $\pm$ QR	222.70 $\pm$ 721.20	268.80 $\pm$ 531.50
Age, years		
Mean $\pm$ SD	39.68 $\pm$ 11.40	38.44 $\pm$ 9.14
Median $\pm$ QR	39.00 $\pm$ 17.50	39.00 $\pm$ 16.50

Values shown in parentheses are percentages.

the last case, the statistical analysis was not significant when comparing the cells incubated with or without IFN- γ . Therefore, it was decided to conduct the ex vivo evaluation using a 24-hour incubation period.

The investigation of MN cells from randomized patients who gave their consent to participate in the ex vivo evaluation demonstrated that IFN- γ apparently downregulated CCR3 mRNA expression ex vivo ($p = 0.068$) and in vivo ($p = 0.043$) in those patients with IgE levels higher than 500 IU/ml. In contrast, IFN- γ upregulated ex vivo ($p = 0.043$) and in vivo ($p = 0.012$) CCR3 mRNA expression in patients with IgE levels lower than 500 IU/ml. Thus, IFN- γ differentially modulated CCR3 mRNA expression ex vivo and in vivo depending on IgE levels. An apparent correlation between ex vivo and in vivo results was observed using this approach (fig. 1).

The results of the evaluation of CCR3 mRNA regulation by IFN- γ were grouped with respect to the regulatory effect exerted by IFN- γ (down- or upregulation), and the average IgE levels of each group were calculated. Figure 2 shows the results of this analysis. IFN- γ downregulated CCR3 mRNA expression in the IgE range approximately between 2,803 and 1,419 IU/ml. Around the 698

Table 2. Clinical evaluation of patients who completed the treatment schedule in our randomized clinical trial with IFN- γ (Heberon Gamma R) and placebo

Variables	Placebo (n = 20)	IFN- γ (n = 19)	Test	p	OR
Crises					
Yes	18 (90.0)	18 (94.7)	Fisher	1.00	2.00 (0.166–24.069)
No	2 (10)	1 (5.3)			
Reduction of crisis number					
High	9 (45.0)	7 (36.8)	χ^2	0.819	0.857 (0.229–3.203)
Moderate	5 (25.0)	7 (36.8)			
Low	2 (10.0)	3 (15.8)			
None	4 (20.0)	2 (10.5)			
Crisis intensity					
Light	5 (27.8)	9 (50.0)	χ^2	0.407	0.583 (0.162–2.097)
Moderate	10 (55.6)	9 (50.0)			
Serious	3 (16.7)	0			
Global evaluation					
Very satisfactory	6 (30.0)	5 (26.3)	χ^2	0.407	0.583 (0.162–2.097)
Satisfactory	4 (20.0)	7 (36.8)			
Regular	7 (35.0)	5 (26.3)			
Bad	3 (15.0)	2 (10.5)			
Symptom persistence					
Improved	3 (15.0)	2 (10.5)	Fisher	0.451	0.438 (0.092–2.083)
Partially improved	11 (55.0)	14 (73.7)			
Without improvement	6 (30.0)	3 (15.8)			

Only 39 patients completed the treatment schedule. Three patients interrupted the treatment. Additionally, 7 patients withdrew voluntarily (3 in the placebo group and 4 in the IFN- γ group), and 1 patient in the IFN- γ group withdrew due to worsening of the condition. Values are numbers of patients, while values shown in parentheses are percentages or 95% confidence intervals, as appropriate. OR = Odds ratio.

IU/ml IgE value, there was no effect of IFN- γ on CCR3 expression, similar to what was found with CCR3 from apparently healthy controls with a mean IgE value of 138 IU/ml. Conversely, in the IgE range between 411 and 351 IU/ml, IFN- γ upregulated CCR3 expression.

An additional interesting observation can be made from the data shown in figure 2. There is an apparently direct correlation between basal (pretreatment) IgE concentrations and CCR3 expression levels. With the decrease in IgE (2,803, 1,646, 1,419, 698, 411, 351 IU/ml), an apparent decline in the relative CCR3 expression is observed (0.67, 0.5, 0.6, 0.42, 0.17, 0.25).

Discussion

Clinical Assessment

A relative overrepresentation of women in the study population was observed. After reviewing several sources of data on the incidence of asthma in Camagüey, Cuba

[32], we found that atopic asthma predominated in women. Participants in the present study were recruited from patients presenting to the Manuel Ascunse Domenech Hospital, where the study was performed. Additionally, it was reported [33] that more than 60% of bronchial asthma patients who received treatment at Family Doctor House 2, polyclinic 'Centro', in Ciego de Avila, Camagüey, were females. These data explain the higher percentage of women enrolled in our clinical trial. Another factor that could influence the difference is that men are less willing to seek medical help for allergic disease than women, and that women are generally more concerned with their health than men [pers. commun., medical doctor allergists].

IFN- γ administration can downregulate the allergic response. In this study, the clinical efficacy of intramuscular IFN- γ in the treatment of asthma was evaluated in a double-blind placebo-controlled clinical trial.

The results obtained in our study with regard to efficacy agree in part with those obtained by Boguniewicz et

Table 3. Identification of the best incubation time to measure the ex vivo regulatory effect of IFN- γ on CCR3 expression (relative levels) in MN cells from asthmatic patients from a previously conducted small pilot study and healthy volunteers

Incubation time	Patients		Controls		p (Mann-Whitney U test)
	MN	MN + IFN- γ	MN	MN + IFN- γ	
2 h					
Number	2	3			0.128
Median $\pm$ QR	0.38 $\pm$ 0.13	0.53 $\pm$ 0.08			
24 h					
Number	6	6	9	8	0.88 (MN cont. vs. MN pat. + IFN- γ cont.)
Median $\pm$ QR	0.49 $\pm$ 0.31	0.25 $\pm$ 0.09	0.29 $\pm$ 0.38	0.24 $\pm$ 0.41	0.077 (MN pat. vs. MN cont.) 0.897 (MN pat. + IFN- γ vs. MN cont.) 0.004* (MN pat. vs. MN pat. + IFN- γ)
48 h					
Number	4	4			
Median $\pm$ QR	0.51 $\pm$ 0.38	0.32 $\pm$ 0.39			0.386
p (Kruskal-Wallis)	0.960	0.222			
* Statistically significant.					

al. [34], who conducted a double-blind placebo-controlled study in which subcutaneous IFN- γ was administered over 90 days in 20 steroid-dependent asthmatic patients, without significant clinical improvement at the end of the study.

In our study, the IFN- γ treatment tended to diminish systemic eosinophilia. Several authors [35–37] have shown that IFN- γ administration slows eosinophilia in the airways as well as systemic eosinophilia. Boguniewicz et al. [34] found a decrease in peripheral eosinophilia in 31% of cases treated with IFN- γ compared to an increase in eosinophilia in 8.5% of the placebo group. Animal models of bronchial atopic asthma where IFN- γ has been administered intranasally, intratracheally, intravenously or in nebulized form have confirmed this IFN- γ -dependent effect.

The decrease in total IgE levels in patients treated with IFN- γ , although not statistically significant, coincides with the data presented by Liu and Xing [38], who investigated the in vitro effect of IFN- γ on IgE synthesis in 30 asthmatic patients and 25 healthy controls. This investigation showed that IFN- γ was capable of suppressing the synthesis of IgE, and that this inhibitory effect was more prominent in the asthmatic patients than in the healthy controls. Furthermore, Teramoto et al. [39] compared the production of IFN- γ by cells stimulated with phytohemagglutinin with serum IgE levels in 20 atopic asthmatic

patients; they found a negative correlation between the levels of serum IgE and the quantity of secreted IFN- γ . The authors concluded that the reduced production of IFN- γ is associated with high levels of IgE in atopic asthmatic patients.

There is much evidence from animal models that IFN- γ downregulates IgE synthesis in vitro [38, 40], but in vivo, the evidence is contradictory [41, 42]. This phenomenon is also reflected in reports of some clinical trials of IFN- γ treatment in atopic patients [43, 44]. This situation could be related to the fluctuating levels of IgE in response to several uncontrolled factors that influence IgE synthesis. Examples of these factors are air pollution and climatic changes, which may have an indirect effect on the allergic response by influencing both quantitatively and qualitatively the pollen production by allergenic plants (seasonal changes) [45], variable levels of endotoxin present in the microenvironment [46] and the use of concomitant therapies [47, 48] that influence the synthesis of IgE. Thus, analysis of the effects of IFN- γ on in vivo IgE levels in asthmatic patients is difficult to follow and interpret.

CCR3 Regulation by IFN- γ

The regulation of chemokine receptors by IFN- γ is well documented in the literature, as reviewed in the Introduction. Most studies in blood have used purified cells, which might give an incomplete view of immune

Fig. 1. Representative ex vivo and in vivo IFN- γ modulation of CCR3 mRNA expression in asthmatic patients enrolled in a randomized double-blind study with IFN- γ and placebo. CCR3 mRNA expression was studied in MN cells isolated from patients before the start of the trial and after ex vivo incubation with or without 500 IU/ml of IFN- γ and after 6 months of treatment with either placebo or IFN- γ , following randomization. (-)IFN = Basal level (before treatment); (+)IFN = incubation ex vivo or treatment in vivo with IFN- γ .

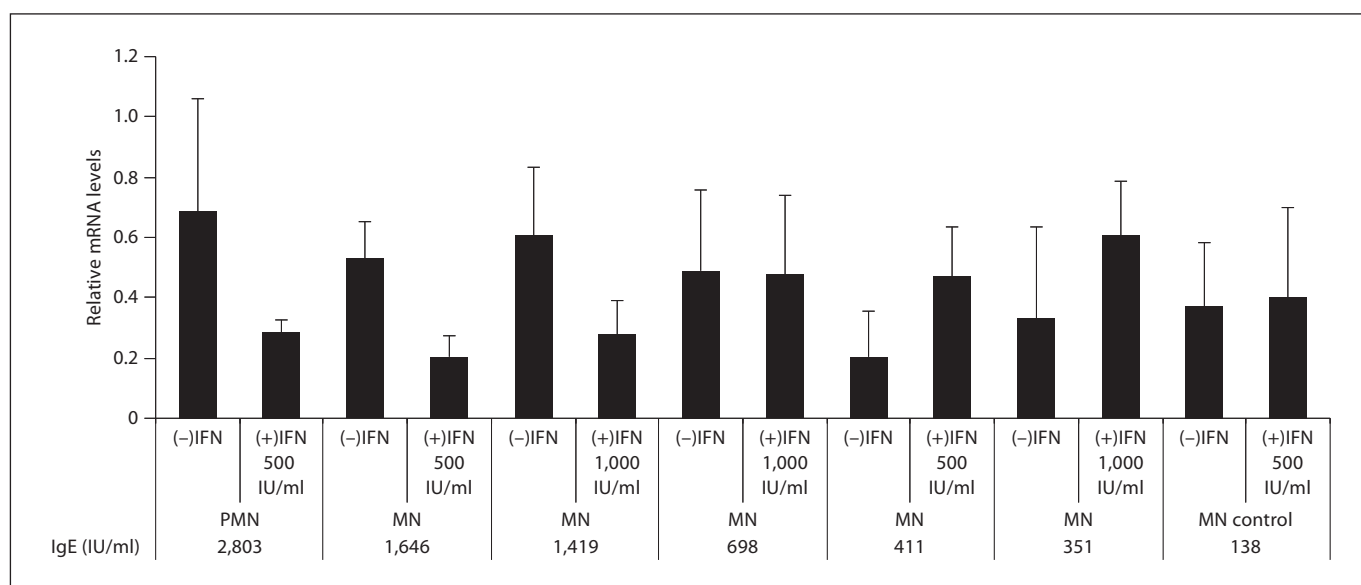
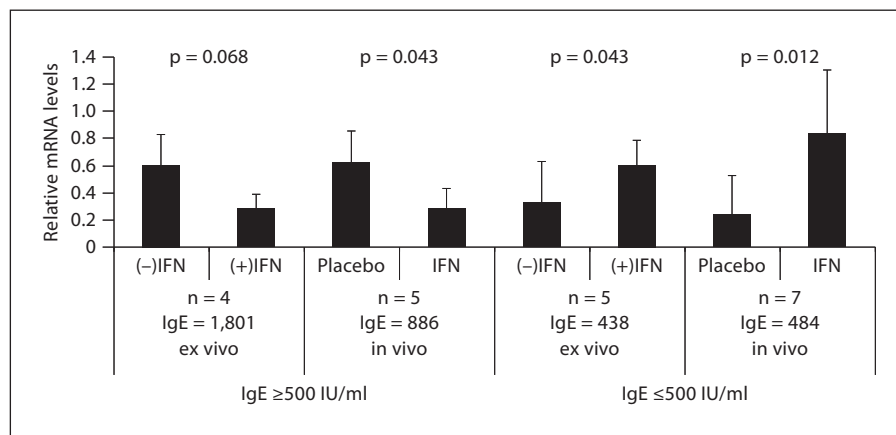


Fig. 2. IFN- γ modulation of CCR3 mRNA expression according to total IgE levels in asthmatic patients. All the results we obtained from patients in the randomized double-blind study with IFN- γ and placebo were used for this analysis, including data from polymorphonuclear (PMN) cells obtained from some patients and data from an ex vivo study in healthy volunteers. CCR3 mRNA

expression was studied in MN or polymorphonuclear cells isolated from patients before the start of the trial and after ex vivo incubation with or without 500 or 1,000 IU/ml of IFN- γ and after 6 months of treatment with either placebo or IFN- γ , following randomization. (-)IFN = Basal level (before treatment); (+)IFN = incubation ex vivo or treatment in vivo with IFN- γ .

reactions. In the current study, we assessed the role of IFN- γ in the regulation of CCR3 in atopic asthma using whole MN cell cultures.

The finding that IFN- γ differentially regulated CCR3 mRNA expression depending on IgE levels offers a new way to try to control the development of asthma, through the regulation of CCR3 expression on CCR3-bearing cells such as eosinophils, mast cells and Th2 lymphocytes, which play a major role in allergic disorders. A sim-

ilar effect has been described for CD23 (low-affinity IgE receptor) regulation by IFN- γ . The effect of IFN- γ on CD23 expression on T cells is dependent on the IgE levels [49]. The modulation of CD23 expression is different on B and T cells, and IFN- γ acts differently in atopic and nonatopic individuals [49]. Allergens also influence the expression of CD23 [49]. In contrast, in the case of CCR3, its expression is apparently independent of allergens [50, 51].

The correlation between the ex vivo and in vivo results in the present study might help to select patients for treatment with IFN- γ who could be expected to respond to the potential therapeutic downregulatory action of IFN- γ on CCR3 expression. The differential downregulation of CCR3 by IFN- γ suggests that this cytokine may be used as a therapeutic approach to control asthma.

The apparent direct correlation between IgE and CCR3 mRNA expression levels noted in this report could be associated with a positive regulatory action of IgE on CCR3 mRNA expression, a process that might enhance the allergic inflammatory cascade. In concordance with our results, Francis et al. [52] recently described an increase in CCR3 expression on T lymphocytes in patients with allergic rhinitis compared with nonallergic controls, and Lun et al. [53] showed that CCR3 levels were higher in asthmatic patients. Moreover, it has been described that mast cells mobilize their intracellular stores of CCR3 for presentation at the cell surface to mediate effector function(s) after IgE-dependent activation [54], likely via activation of several transcription factors (GATA, nuclear factor- κ B) [55] stimulated by IgE [56, 57] acting on the

CCR3 promoter. Now, our data indicate that this process could also be a characteristic of peripheral MN cells. The interplay between IgE and IFN- γ signaling might be at the core of these different behaviors.

However, the clinical outcome in the present study did not reflect a statistically significant benefit with IFN- γ treatment, although the IFN- γ group did always show better clinical results than the placebo group. This was most evident with regard to controlling crisis intensity. Thus, in light of the fact that there is a subset of patients who are susceptible to downregulation of CCR3 by IFN- γ , and taking into account that IFN- γ upregulates CCR5 [19, 20], expressed mainly in the Th1 phenotype, a significant therapeutic effect with systemic IFN- γ might be expected in this subset of asthmatic patients.

Acknowledgments

We thank Dr. M. Loza (Department of Internal Medicine, Center for Human Genomics, USA) for critical comments, suggestions and reading of the manuscript. The authors received funds from government sources.

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