

The effect of cetirizine on IFN- γ and IL-10 production in children with allergic rhinitis

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Cetirizine, one of the most commonly used antihistamines for the treatment of allergic diseases, possesses some anti-inflammatory properties. Despite its common use, the effect of cetirizine on the production of cytokines from peripheral blood mononuclear cells (PBMCs) needs further clarification. The aim of this study was to investigate whether cetirizine changes interleukin (IL)-10, (IFN)- γ and IL-4 production from PBMCs in children with allergic rhinitis.

Thirteen children with allergic rhinitis sensitized to house dust mite (HDM) were treated with cetirizine for four weeks. Blood samples were drawn just prior to the treatment, on the last day of the treatment and two weeks following the cessation of treatment. The cytokine production from PBMCs was tested in the presence or absence of HDM allergen and measured by ELISA assay.

An augmentation in IL-10 production was observed in PBMCs at the 4th week of cetirizine treatment ($p < 0.05$). Furthermore, a significant increase in IFN- γ production was observed following the therapy. IL-4 release did not change at all time points tested. In addition, IFN- γ /IL-4 ratio increased following cetirizine treatment.

Cetirizine induced a shift in the human Th1/Th2 cytokine balance toward a Th1 type response by increasing IFN- γ production and augmenting suppressor cytokine release (IL-10). We concluded that apart from its known antihistaminic properties, cetirizine may modulate allergic inflammation while the patients are on regular treatment schedules.

Key words: allergic rhinitis, cetirizine, IFN- γ , IL-10, IL-4.

The inflammatory reaction characteristic of allergic rhinitis occurs in two stages: an early-phase response and a late-phase response. Early-phase response typically occurs following the interaction of an allergen with immunoglobulin E, and is characterized by the release of certain mediators. Histamine, one of the important mediators of early-phase response, causes mucus hypersecretion, increased vascular permeability and mucosal edema, which may result in rhinorrhea, nasal congestion, itching and sneezing. Following the early phase of the allergic response, many patients demonstrate a late-phase reaction characterized by cell adhesion molecule over-expression, release of inflammatory mediators

with chemotactic activity and cytokine production¹⁻³. Allergic inflammation has been linked to an excessive production of the Th2 cytokines interleukin (IL)-4 and IL-5 relative to the Th1 cytokine interferon (IFN)- γ ⁴. IL-10, a regulatory/suppressor cytokine^{5,6}, has an important role in the regulation of allergic immune responses by facilitating T-cell tolerance and prevention of tissue inflammation^{7,8}. Antihistamines are the first-line medications generally used for the symptomatic treatment of allergic disorders. These drugs, in addition to their antihistaminic properties, also have some additional anti-inflammatory effects⁹⁻¹². Cetirizine, an H1-antihistamine, demonstrates some anti-inflammatory properties by reducing late phase

allergic responses through the inhibition of leukocyte recruitment and by decreasing intercellular adhesion molecule (ICAM)-1 expression¹³⁻¹⁶. Although some anti-inflammatory properties of cetirizine have been reported in many in vitro and animal studies, it is not clear whether these effects could change the balance of the allergic inflammatory mechanism while the patients are on regular treatment schedules. To our knowledge, this is the first study aiming to investigate whether certain cytokine production of peripheral blood mononuclear cells (PBMCs) is affected by regular cetirizine treatment in children with allergic rhinitis. Therefore, we investigated IFN- γ , IL-4 and IL-10 production of PBMCs in children with allergic rhinitis before, during and after cetirizine treatment.

Material and Methods

Subjects

Thirteen newly referred children with perennial allergic rhinitis (7 females and 6 males) were included in the study. Each patient was required to demonstrate at least a none-year history of mild to moderate perennial allergic rhinitis. All patients were selected on the basis of history, clinical findings and allergy to house dust mite (HDM) (*Dermatophagoides pteronyssinus*). HDM allergy was defined both by a positive skin prick test response (a wheal response 3 mm greater than negative control) and *Dermatophagoides pteronyssinus* specific IgE level of greater than 50 ku/L (UniCAP System, Pharmacia, Sweden). The patients were then put on cetirizine treatment (5 mg for <30 kg and 10 mg for >30 kg, Zyrtec, UCB Pharma) for a duration of four weeks. If the patients received any other drug for any reason, they were excluded from the study. Venous blood samples were obtained just before the treatment (baseline), on the last day of the treatment (4th week) and two weeks after the cessation of cetirizine therapy (6th week). All parents provided informed consent and the study protocol was approved by the Ethics Committee of Akdeniz University.

PBMC Culturing

Peripheral blood mononuclear cells were isolated through Ficoll-Hypaque density gradient centrifugation, washed twice with phosphate buffered solution (PBS) and

resuspended in RPMI 1640. After adjusting the cell number to 1×10^6 in 1 ml of media, PBMCs were stimulated with HDM extract at the final concentration of 100 μ g of protein/ml. Parallel cultures were established in order to test if PBMCs were stimulated upon allergen exposure and were able to release cytokines. Therefore, negative control cultures were incubated without the allergen and represented unstimulated resting PBMCs. PBMCs were incubated for six days at 37°C on 5% carbon dioxide. Culture supernatants were obtained from parallel cultures of stimulated and resting cells after six days of HDM stimulation.

Allergens

Lyophilized HDM extracts were obtained from Dr. Cromwell (allergopharma Joachim Ganzer Kg, Hamburg). HDM extract was reconstituted by extracting 1 g of powder in 10 ml of RPMI by mixing overnight at 4°C, followed by centrifugation and filter sterilization of the supernatant through a 0.2 micron filter.

Cytokine Analyses

Interleukin IL-10, IFN- γ and IL-4 levels of culture supernatants were measured using a commercial ELISA kit (Immunotech, Beckman Coulter Company, France). The results were calculated by interpolation from a standard curve that was constructed in the same assay.

Statistical Analyses

All data were expressed as mean \pm SE. SPSS program 10.0.1 was used to analyze the data. Friedman and Wilcoxon signed ranks tests were used to compare cytokine release from PBMCs using samples representing three different stages of cetirizine treatment. A p value of less than 0.05 was considered statistically significant.

Results

Results are summarized in Table I.

Comparison of Cytokine Release of PBMCs in parallel Cultures

As expected, allergen stimulation caused increases in cytokine production (IL-10, IFN- γ and IL-4) of PBMCs compared to resting cells at all time points tested (0, 4th and 6th weeks). Although allergen stimulation caused an increase in IL-4 production at the 4th week of treatment, this increase was statistically insignificant (Table I).

Table I. Comparison of Cytokine Release of PBMCs in Parallel Cultures at Different Stages of Cetirizine Treatment

Parallel cultures	Baseline (0)		4 th week		6 th week	
	Resting	Stimulated	Resting	Stimulated	Resting	Stimulated
IL-100 (pg/ml)	36.5 \pm 4.6	188.1 \pm 53.9*	58.8 \pm 8.8†	294.2 \pm 98.7*	37.7 \pm 98.7*	381.9 \pm 156.0
IFN- γ (IU/ml)	1.9 \pm 0.3	3.3 \pm 0.5*	1.7 \pm 0.3	2.8 \pm 0.6*	1.5 \pm 0.3	12.9 \pm 2.8*¶†
IL-4 (pg/ml)	20.6 \pm 1.9	28.9 \pm 4.7*	20.8 \pm 2.3	25.7 \pm 2.9	17.9 \pm 1.5	24.2 \pm 2.6*

Data is shown as mean \pm SE.

PBMC: peripheral blood mononuclear cells; IL: interleukin; IFN: interferon.

* $p < 0.05$; versus resting culture values.

¶ $p < 0.05$; versus 4th week values.

† $p < 0.05$; versus baseline values.

Cytokine Release of Resting PMCs

Interferon IFN- γ production of resting cells at the 4th and 6th week of treatment was not different from baseline levels ($p > 0.05$ for each; Fig. 1). Cetirizine increased IL-10 production of resting PBMCs (from 36.5 \pm 4.6 pg/ml to 58.8 \pm 8.8 pg/ml) at the 4th week of treatment compared to samples taken pre-treatment ($p < 0.05$; Fig. 2). IL-4 production in resting PBMCs did not change at all time points tested ($p > 0.05$ for each; Fig. 3).

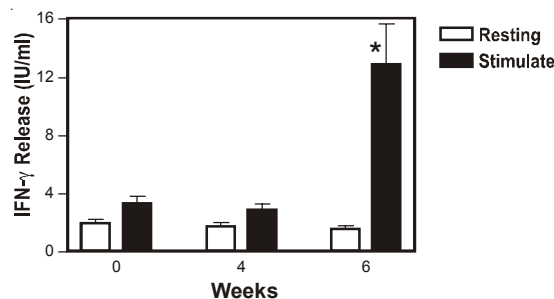


Fig. 1. IFN- γ production of peripheral blood mononuclear cells *: $p < 0.05$.

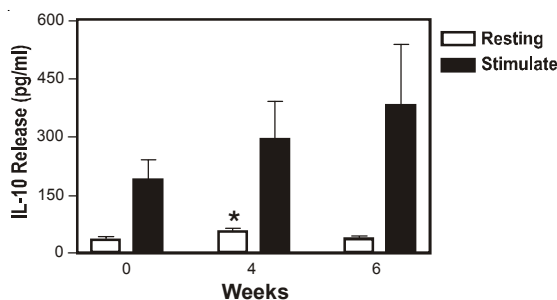


Fig. 2. IL-10 production of peripheral blood mononuclear cells *: $p < 0.05$.

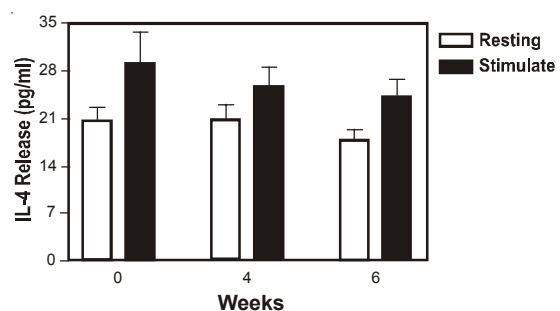


Fig. 3. IL-4 production of peripheral blood mononuclear cells.

Cytokine Release of Stimulated PBMCs

After stimulation with HDM allergen, IFN- γ levels were measured as 3.3 \pm 0.5, 2.8 \pm 0.6 and 12.9 \pm 2.8 IU/ml at weeks 0, 4 and 6, respectively (Fig. 1). HDM-induced IFN- γ release at the 6th week of therapy was higher than both pre-treatment (week 0) and 4th week values ($p < 0.05$ for each). Although an increase in HDM allergen-induced IL-10 release was also observed at week 4, this change was statistically insignificant (Fig. 2). There was no alteration in terms of IL-4 production (Fig. 3).

Th1/Th2 Cytokine Ratio in the Culture Supernatants of PBMCs

Cytokine release of PBMCs was also analyzed as the ratio of the IL-4 (in pg/ml) to IFN- γ (in IU/ml) in each culture. This ratio is expressed as a Th1/Th2 cytokine ratio in arbitrary unit. We observed that IFN- γ /IL-4 ratio at the 6th week (0.61) was significantly higher than that observed at 0 (0.11) and 4th week (0.14) in the culture supernatants of stimulated PBMCs ($p < 0.05$ for each). Th1/Th2 cytokine ratio for the resting PBMCs was not altered at all time points tested (Fig. 4).

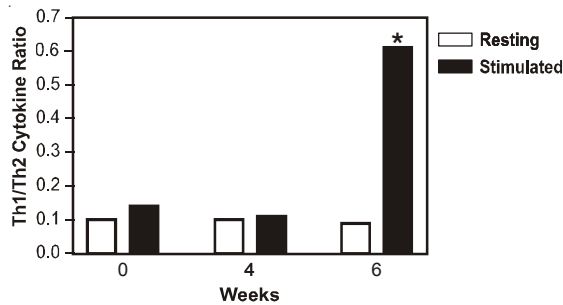


Fig. 4. Th1/Th2 cytokine ratio in the culture supernatants of peripheral blood mononuclear cells
*:p<0.05.

Discussion

We have shown that cetirizine caused an increase in IFN- γ levels and Th1/Th2 cytokine ratio in the HDM-stimulated culture supernatants of PBMCs (Figs. 1, 4). Based on this observation, we concluded that cetirizine itself shifted the balance of Th1/Th2 type cytokine of human PBMCs toward a predominant Th1 type response. We further observed a significant increase in IL-10 production of resting PBMCs following the four-week treatment (Fig. 2).

Cetirizine, one of the new generation antihistamines, is considered safe for prolonged use with only a few side effects¹⁷. Additionally, cetirizine is well tolerated and effective in relieving the symptoms of allergic rhinitis^{18,19}. Beyond its antihistaminic activity, cetirizine might exert some additional antiallergic effects, such as the inhibition of the up-regulation of ICAM-1/CD 54, eosinophil recruitment, and the release of prostaglandin D₂, leukotrienes and PAF^{13-16,20}.

However, there are a limited number of studies investigating the effects of cetirizine on the release of cytokines, which have an important role in allergic inflammation. Furthermore, these studies were generally based on in vitro studies and gave controversial results about Th1 and Th2 responses^{9,10,14,15}. Considering the complexity of the patho-physiology of allergy in humans, we believe that the effect of antihistamines on cytokines should be investigated by in vivo studies. As far as we know, the effect of antihistamines on cytokine production has not been investigated previously by in vivo studies. Cytokines are generally produced locally and mainly have paracrine effects. Although we did not study the

mononuclear cells from the nasal lymphoid tissue, we investigated the effect of cetirizine on cytokine production during a routine treatment. Therefore, it would be presumptuous to claim that the same changes in cytokine dynamics are true at the microenvironment of nasal tissue in humans.

Human T-helper subsets were based on the relative proportions of Th1 and Th2-type cytokines produced by T-cell clones. An excess of Th2 cytokines relative to the Th1 cytokine IFN- γ , rather than the amounts of these cytokines, is implicated in the cause of atopy^{21,22}. We analyzed the ratio of the IFN- γ to IL-4 and expressed this ratio as a Th1/Th2 cytokine ratio, because PBMCs contain Th1, Th2 and Th0 cells and cytokine release cannot be used to determine the phenotype of the cells. Studies on allergen-stimulated PBMCs reported decreased IFN- γ release in subjects with atopic diseases relative to healthy control subjects. In our study, we observed an increase in both IFN- γ production and Th1/Th2 cytokine ratio in allergen-stimulated culture of PBMCs after cetirizine treatment in children with allergic rhinitis.

We also found a significant increase in IL-10 release of resting PBMCs at the 4th week of the cetirizine treatment (Fig. 2). IL-10 is known as a suppressor cytokine and major regulatory agent of inflammatory responses. IL-10 is a general inhibitor of T-cell proliferation and cytokine response. It is mainly produced by mononuclear cells, natural killer cells, and Th1- and Th2-type lymphocytes^{5,6}. IL-10 levels have been reported to be reduced in an asthmatic airway, potentially contributing to more intense inflammation⁸. Furthermore, IL-10 was previously reported to inhibit IFN- γ synthesis²³. The stimulatory effect of cetirizine on IFN- γ levels might be delayed due to the early increase in IL-10 production. The drug-induced alterations in certain cytokine production may also demonstrate different kinetics.

Since cetirizine induced a shift in the Th1/Th2 balance toward a Th1 type response and a stimulatory effect on the production of suppressor cytokine IL-10, it is considered to have anti-inflammatory properties, as suggested in many other studies. This inhibitory effect on the allergic mechanism, in addition to its antihistaminic properties, could be an explanation for the beneficial effects of cetirizine

in atopic diseases. Based on our results and others, it can be speculated that cetirizine may modulate the allergic inflammation during the treatment of allergic diseases when it is used at conventional doses. In conclusion, further studies are necessary to better understand whether cetirizine-induced increase in Th1/Th2 ratio or augmentation of IL-10 release has any additional clinical effect.

REFERENCES

1. Naclerio RM. Allergic rhinitis. *N Engl J Med* 1991; 325: 860-869.
2. Skoner DP. Allergic rhinitis: definition, epidemiology, pathophysiology, detection and diagnosis. *J Allergy Clin Immunol* 2001; 108: S2-S8.
3. Naclerio RM, Proud D, Togias AG, et al. Inflammatory mediators in late antigen-induced rhinitis. *N Engl J Med* 1985; 313: 65-70.
4. Mosmann TR, Sad S. The expanding universe of T-cell subsets: Th1, Th2 and more. *Immunol Today* 1996; 17: 138-146.
5. Fiorentino DF, Zlotnik A, Mosman TR, Howard M, O'Garra A. IL-10 inhibits cytokine production by activated macrophages. *J Immunol* 1991; 147: 3815-3822.
6. De Wall Malefyt R, Abrams J, Bennett B, Figdor CG, De Vries JE. Interleukin 10 (IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes. *J Exp Med* 1991; 174: 1209-1220.
7. Belinghausen I, Knop J, Saloga J. The role of interleukin 10 in the regulation of allergic immune responses. *Int Arch Allergy Immunol* 2001; 126: 97-101.
8. Takanashi S, Hasegawa Y, Kanehira Y, et al. Interleukin-10 level in sputum is reduced in bronchial asthma, COPD and in smokers. *Eur Respir J* 1999; 14: 309-314.
9. Lippert U, Moller A, Welker P, Artuc M, Henz BM. Inhibition of cytokine secretion from human leukemic mast cells and basophils by H1- and H2-receptor antagonists. *Exp Dermatol* 2000; 9: 118-124.
10. Kaminuma O, Ogawa K, Kikkawa H, Kikuchi M, Naito K, Ikezawa K. A novel anti-allergic drug, betotastine besilate, suppresses interleukin-5 production by human peripheral blood mononuclear cells. *Biol Pharm Bull* 1998; 21: 411-413.
11. Benlounes N, Dupuyont C, Candalh C, Blaton M-A, Bloom M, Heyman M. Effect of terfenadine on TNF- α release from peripheral blood mononuclear cells during cow's milk allergy. *Clin Exp Allergy* 1997; 27: 942-948.
12. Charlesworth EN, Massey WA, Kagey-Sobotka A, Norman PS, Lichtenstein LM. Effects of H1 receptor blockade on the early and late response to cutaneous lallergen challenge. *J Pharmacol Exp There* 1992; 262: 964-970.
13. Mincarini M, Cagnoni F, Canonica GW, et al. Quantitative flow cytometric analysis of the effects of cetirizine on the expression of ICAM-1/CD54 on primary cultured nasal cells. *Allergy* 2000; 55: 226-231.
14. Jin Hr, Okamoto Y, Matsuzaki Z, Endo S, Ito E. Cetirizine decreases interleukin-4, interleukin-5, and interferon-gamma gene expressions in nasal-associated lymphoid tissue of sensitized mice. *Am J Rhinol* 2002; 16: 43-48.
15. Arnold R, Rhioux J, Konig W. Cetirizine counter-regulates interleukin-8 release from human epithelial cells (A549). *Clin Exp Allergy* 1999; 29: 1593-1596.
16. Townley RG, Okada C. Use of cetirizine to investigate non-H1 effects of second-generation antihistamines. *Ann Allergy* 1992; 68: 190-196.
17. Simons FE. Prospective, long-term safety evaluation of the H1-receptor antagonist cetirizine in very young children with atopic dermatitis. ETAC study Group. Early Treatment of the Atopic Child. *J Allergy Clin Immunol* 1999; 104: 433-440.
18. Mansmann HC Jr, Altman RA, Berman BA, et al. Efficacy and safety of cetirizine therapy in perennial allergic rhinitis. *Am Allergy* 1992; 68: 348-353.
19. Noonan MJ, Raphael GD, Nayak A, et al. The health-related quality of life effects of once-daily cetirizine HCL in patients with seasonal allergic rhinitis: a randomized double-blind, placebo-controlled trial. *Clin Exp Allergy* 2003; 33: 351-358.
20. Kalaycı O, Saraçlar Y, Adalıoğlu G, Sekerel B, Tuncer A. The effect of cetirizine on sulfidoleukotriene production by blood leukocytes in children with allergic rhinitis. *Allergy* 1995; 50: 964-969.
21. Romagnani S. Lymphokine production by human T cells in disease states. *Annu Rev Immunol* 1994; 12: 227-257.
22. Umetsu DT, DeKruyff RH. T_H1 and T_H2 CD4⁺ cells in human allergic diseases. *J Allergy Clin Immunol* 1997; 100: 1-6.
23. Hsu DH, Moore KW, Spits H. Differential effects of IL-4 and IL-10 on IL-2-induced IFN-gamma synthesis and lymphokine-activated killer activity. *Int Immunol* 1992; 4: 563-569.