

Airway inflammation and eotaxin in exhaled breath condensate of patients with severe persistent allergic asthma during omalizumab therapy

Zietkowski Z*, Skiepmo R, Tomasiak-Lozowska MM, Bodzenta-Lukaszyk A

Department of Allergology and Internal Medicine, Medical University of Białystok, Poland

* CORRESPONDING AUTHOR:

Department of Allergology and Internal Medicine,
Medical University of Białystok,
Sklodowska-Curie Street 24A,
15-276 Białystok, Poland.
tel. 0048-85-7468373, fax. 0048-85-7468601
e-mail: z.zietkowski@wp.pl (Ziemowit Zietkowski)

Received 10.11.2010

Accepted 05.05.2011

Advances in Medical Sciences

Vol. 56 • 2011

DOI: 10.2478/v10039-011-0024-0

© Medical University of Białystok, Poland

ABSTRACT

Purpose: The central role of IgE in allergic inflammation in asthma has provided a rationale for the development of omalizumab, the humanized monoclonal anti-IgE antibody. The aim of the study was to determine the effect of omalizumab treatment on changes in airway inflammatory process and eotaxin in exhaled breath condensate in patients with persistent severe allergic asthma.

Material and Methods: The study was performed on a group of 19 patients with severe persistent allergic asthma treated with conventional therapy (according to GINA 2006) and with or without omalizumab (9 vs 10 patients). Eotaxin in exhaled breath condensate, exhaled nitric oxide, blood eosinophil count and serum ECP were measured before and after 16 weeks of therapy.

Results: In the group treated with omalizumab, a statistically significant decrease in the concentrations of eotaxin in EBC, FENO, serum ECP, and blood eosinophil count after 16 weeks of treatment was observed. Statistically significant correlations were revealed between the decrease in eotaxin and the decrease in FENO, serum ECP and blood eosinophil count after omalizumab therapy.

Conclusions: Downregulation of eotaxin expression in the airways through limitation of eosinophilic inflammation could be essential in the beneficial effect of anti-IgE therapy with omalizumab in asthma patients.

Key words: omalizumab, asthma, eotaxin, airway inflammation, exhaled breath condensate

INTRODUCTION

Omalizumab is a monoclonal anti-immunoglobulin E antibody developed for the treatment of asthmatic patients with inadequately controlled moderate to severe persistent allergic asthma despite optimal controller therapy [1].

Eosinophils have a well characterized role in asthmatic airway inflammation. The presence of eosinophils in airways is the consequence of a multistep and multifactorial process. Interactions of eosinophils with endothelial cells through adhesion molecules, and local generation of chemotactic agents involved in cell migration into the inflamed airways, are recognized as important mechanisms in the pathophysiology of airway inflammation [2]. The key factors affecting the increase in eosinophil chemotaxis to the site of inflammation as well as the prolonging of their survival, are IL-3, IL-5, and

GM-CSF [3]. These act together with selective chemokines of eosinophils, such as eotaxin, RANTES, or MCP-4 (monocyte chemoattractant protein-4). The strongest and the most specific chemoattractant is eotaxin [4, 5].

Mast cells and basophils are key cells connected with omalizumab activity. Mast cells have been closely associated with the cumulating inflammatory cells (including eosinophils), mucosal inflammation, endothelial activation, and bronchial hyperresponsiveness, through the release of cytokines such as Th2 cytokines, including interleukins 4 and 5 (IL-4, IL-5) and tumor necrosis factor – α (TNF- α) [6].

The mechanisms underlying the clinical and anti-inflammatory efficacy of omalizumab are not fully understood. There is no data concerning the effect of anti-immunoglobulin E treatment on eotaxin expression in the asthmatic airways.

The aim of the study was to assess changes in eotaxin

Table 1. Characteristics of studied patients.

Characteristics	Dimension	Patients treated with omalizumab	Patients without omalizumab	Differences between groups of asthma patients
Number of patients		9	10	
Sex	F/M	1/8	3/7	
Age	Years	49.3±8.2	52.0±8.6	p=0.95
Duration of symptoms	Years	13.4±8.1	15.3±12.5	p=0.67
Baseline FEV ₁	% predicted	49.2±10.8	51.4±8.8	p=0.63
Blood eosinophil count	cells/mm ³	294.4±283.8	170.3±72.8	p=0.14
Baseline F _{ENO}	ppB	59.4±46.8	41.0±26.6	p=0.30
Serum total IgE	kU/L	218±123	156±150	p=0.36
eotaxin (EBC)	pg/ml	19.37±3.98	17.28±2.83	p=0.33
ECP (serum)	mcg/l	25.6±18.4	16.6±6.1	p=0.16
ICS dose ^x	mg/day	1138±253	1125±317	p=0.86

Data are presented as medians (ranges)

FEV₁ - forced expiratory volume in one second

F_{ENO} - exhaled nitric oxide

ECP - eosinophil cationic protein

^xInhaled corticosteroids (Fluticasone propionate equivalent)

concentrations in the exhaled breath condensate (EBC) of asthmatics with severe persistent allergic asthma during omalizumab therapy as well as to establish the possible correlation of these measurements with other parameters of airway inflammation.

MATERIAL AND METHODS

Patients

The study was conducted on a group of 19 patients with severe persistent allergic asthma.

All patients used inhaled short-acting β_2 -agonists (as rescue medication), inhaled long-acting β_2 -agonists, high-doses of inhaled steroids (without oral steroids), and leukotriene receptor antagonists. It was an open-label study. Randomization was not performed. Nine patients with severe persistent allergic asthma who met criteria to receive omalizumab therapy were assigned to omalizumab therapy. Subsequently, 10 patients with severe allergic persistent asthma who met criteria to receive omalizumab therapy were assigned to the group treated with conventional treatment - recommended by GINA 2006 [7], without omalizumab. The authors have taken efforts to make the two studied groups comparable regarding the severity and kind of asthma, spirometric indices, intensity of day and night symptoms, and the controlling treatment used, as well as the necessity for rescue medication and other parameters used in asthma control assessment. All the patients were atopic and sensitized to common inhaled allergens, as evaluated by skin prick tests; they had total serum IgE of at least 30 to no more than 700 IU/ml. All patients had been in a stable condition, free from acute exacerbations and respiratory tract infections for the previous two months. They were non-smokers and

during the last year had not been passive smokers.

Patients were treated with omalizumab on the basis of the concentration of serum total IgE and patient body weight at baseline [8]. The study protocol was approved by the Ethics of Research Committee of the Medical University of Bialystok, agreement number: R-I-002/68/2007. Informed consent was obtained from every patient entered into the study.

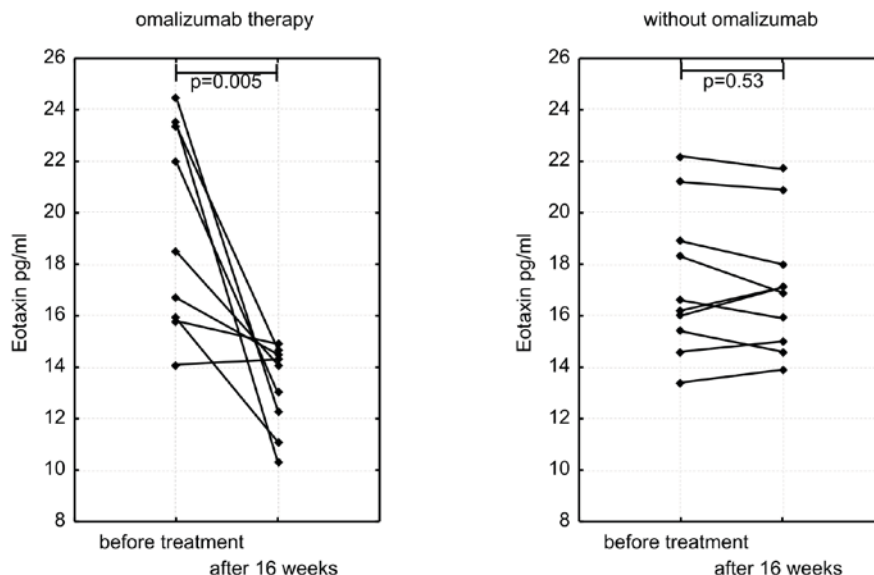
Measurements

Studied parameters were measured before and after a 16 week treatment period.

The measurements of exhaled nitric oxide (F_{ENO}) were performed by the chemiluminescence technique using a Sievers 280i NO Analyzer (Boulder, Colorado, USA). The measurements were performed on-line at an expiratory flow of 50ml/s [9]. Spirometry was performed according to ATS standards using a MasterScreen Pneumo PC spirometer (Jaeger, Hoechberg, Germany) [10].

Exhaled breath condensate was collected by using a commercially available condenser (EcoScreen; Erich Jaeger GmbH, Hoechberg, Germany) according to current ATS/ERS guidelines [11]. The longest storage time of EBC samples did not exceed two months. The samples were not concentrated prior to measurement. Because the marker used to correct the difference in the degree of dilution has not yet been established, in our study we made no attempt to assess the dilution of ALF in EBC. The results were well repeatable {CV(%)= 4-7%}. We performed the preliminary study, in which we measured eotaxin in EBC immediately after collection and after 1, 2, and 3 months of storage at -80°C, and did not observe important changes. The concentrations of eotaxin (R&D Systems, Wiesbaden-Nordenstadt, Germany) in EBC were determined using an enzyme-linked immunosorbent assay. The minimum detectable level was 5.0 pg/ml.

Figure 1. Concentrations of eotaxin in EBC before the treatment, and subsequent changes which were observed after 16 weeks in both groups of asthmatic patients.



Serum total IgE concentrations and serum ECP were measured using ImmunoCAP™ Technology (Pharmacia Diagnostics, Uppsala, Sweden). The minimum detectable level of ECP was 2.0 µg/l. Blood eosinophil count was measured using a hematologic analyzer (Coulter Electronics GmbH, Miami, Florida, USA).

Analysis

Statistical significance was analyzed by using analysis of variance (ANOVA) followed by Bonferroni's t test post hoc to determine statistical differences. All values were expressed as means ± SD; p values < 0.05 were considered significant. The relationship between studied parameters was assayed by correlation. Pearson's linear correlation coefficient was used.

RESULTS

Characteristics of patients are presented in table 1 (Tab. 1).

In the group of patients treated with omalizumab after 16 weeks of treatment, we found a statistically significant decrease in eotaxin levels in EBC (before therapy: 19.37±3.98pg/ml, after therapy: 13.24±1.67, p=0.005) (Fig. 1) and F_{ENO} (before therapy: 59.4±46.8ppB, after therapy: 26.43±18.2, p=0.01). We also observed a statistically significant decrease in serum ECP (before therapy: 25.6±18.4µg/l, after therapy: 10.41±3.45, p=0.02) and blood eosinophil count (before therapy: 294.4±283.8, after therapy: 136.6±114.5, p=0.04).

We did not observe such changes in the group treated with conventional treatment without omalizumab (eotaxin - before therapy: 17.28±2.83pg/ml, after therapy: 17.11±2.55, p=0.53; F_{ENO} - before therapy: 41.0±26.6ppB, after therapy: 34.8±20.16, p=0.06; serum ECP - before therapy: 16.6±6.1µg/l, after

Table 2. Correlations between changes observed in studied parameters after 16 weeks of therapy.

Studied parameters	FENO	ECP	Eos
Patients treated with omalizumab.			
Eotaxin (pg/ml)	r=0.99 p<0.0001	r=0.68 p=0.04	r=0.68 p=0.04
F_{ENO} (ppB)		r=0.63 p=0.04	r=0.69 p=0.03
ECP (mcg/l)			r=0.79 p=0.01
Patients treated without omalizumab.			
Eotaxin (pg/ml)	r=0.009 p=0.98	r=0.24 p=0.48	r=0.001 p=0.99
F_{ENO} (ppB)		r=0.4 p=0.24	r=0.58 p=0.08
ECP (mcg/l)			r=0.6 p=0.05

therapy: 16.3±5.68, p=0.48; blood eosinophil count - before therapy: 170.3±72.8, after therapy: 161.6±80.5, p=0.97).

In the group of patients treated with omalizumab, we found a statistically significant correlation between the decrease in eotaxin and the decrease in F_{ENO} , serum ECP and blood eosinophil count after therapy. Such correlations were not observed in the group of patients treated without omalizumab (Tab. 2).

DISCUSSION

Eosinophils have a well characterized role in the intensification of airway inflammation in asthmatic patients through the release of a variety of pro-inflammatory mediators, including granule-

stored cationic proteins, newly synthesized eicosanoids, and cytokines [12,13].

Omalizumab is a recombinant monoclonal humanized anti-immunoglobulin E antibody. It is used in the treatment of patients with poorly controlled severe persistent allergic asthma despite optimal controller therapy [1]. By reducing serum IgE levels and expression of FcεRI receptor on key cells in the inflammatory cascade, omalizumab blocks the release of inflammatory mediators from mast cells and reduces the infiltration of inflammatory cells, especially eosinophils, into the airways [14].

The clinical efficacy of omalizumab has been demonstrated in randomized controlled clinical trials [15]. Several studies have shown that treatment with omalizumab reduces both peripheral and bronchial tissue eosinophil counts, as well as diminishes the release of a variety of pro-inflammatory mediators such as GM-CSF, IL-2, 4, 5, and IL-13 [16,17].

The mechanisms underlying the clinical efficacy of omalizumab are not fully understood and in asthmatic patients there is no data concerning the effect of omalizumab therapy on eotaxin, which is the strongest chemotactic factor for eosinophils and one of the main mediators taking part in the development of eosinophil infiltration and allergic inflammation.

The effect of omalizumab therapy was also estimated in other than asthma diseases. Foroughi et al demonstrate that IgE-mediated processes contribute to the generation of eosinophilic inflammation in eosinophil associated gastrointestinal disorders. They show that omalizumab therapy is associated with significant decrease in peripheral eosinophilia and gastrointestinal symptoms, and a trend towards lower eosinophil number in the gastric antrum and duodenum [18].

In this study we have found that, in patients with severe asthma, anti-IgE therapy with omalizumab statistically significantly decreases the concentration of eotaxin in EBC. This decrease significantly correlates with a decrease in other studied immunological parameters such as exhaled nitric oxide, serum ECP and blood eosinophil count.

The current study demonstrates a connection between anti-IgE treatment and eotaxin expression in the airways. Eotaxin can be produced by many human cells, such as airway epithelium, endothelial cells, lymphocytes, macrophages, and eosinophils, as well as airway smooth muscle cells [5]. All these cells are present in the airways and many of them are strongly involved in asthmatic inflammation. Eotaxin is recognized as the strongest and most specific factor, affecting eosinophil function. This chemokine causes the release of progenitors of eosinophils from the bone marrow. Acting together with IL-5, eotaxin could induce the increase in the count of mature eosinophil forms in peripheral blood. The result of all these processes is peripheral and tissue eosinophilia [19]. The count of eosinophils in the infiltrated organ depends on eotaxin concentration in this site. This chemokine is also involved in the regulation of the life cycle of eosinophils, by taking part in

the retardation of their apoptosis [5]. The interaction between eotaxin and its receptor on eosinophils leads to their activation and degranulation [5]. Moreover, eotaxin can elicit migration of mast cells [20] and basophils [21].

The mechanisms of antiinflammatory actions of anti-IgE treatment include the limitation of IgE action on effector cells, such as mast cells and basophils, and inhibition of the accumulation of inflammatory cells into the airways, as well as a decrease in the release of Th2 cytokines [22]. In our previous publications we described that during anti-immunoglobulin E therapy with omalizumab in patients with severe persistent allergic asthma, RANTES and endothelin – 1 expression in the airways (measured in exhaled breath condensate) were decreased [23,24].

On the surface of the cells, which can produce eotaxin, FcεRII - low affinity receptors for IgE are expressed, which play a minimal role in the biological activity of IgE. High affinity receptors for IgE, FcεRI, which play an important role in IgE-mediated allergic reactions, are present on mast cells and basophils, which are not a potent source of eotaxin [6]. Therefore, the effect of anti-IgE therapy with omalizumab on decreased expression of eotaxin in the airways seems to be mainly connected with the limitation of production of many proinflammatory cytokines (e. g. IL-4 and IL-13), which stimulate inflammatory cells to increased expression and release of this chemokine [25,26].

CONCLUSIONS

This is the first study in which it was revealed that, in asthmatic patients, anti-IgE therapy with omalizumab causes a decrease in expression of eotaxin in exhaled breath condensate. This may in turn lead to limiting eosinophil recruitment and activation and could be one of the key elements responsible for the beneficial effect of anti-IgE therapy with omalizumab.

ACKNOWLEDGEMENTS

The authors declare that they have no competing interests in the publication of the manuscript. This work was supported by research grant No 3-06732P from the Medical University of Białystok, Poland.

REFERENCES

1. Ayres JG, Higgins B, Chilvers ER, Ayre G, Blogg M, Fox H. Efficacy and tolerability of anti-immunoglobulin E therapy with omalizumab in patients with poorly controlled (moderate-to-severe) allergic asthma. *Allergy*. 2004 Jul;59(7):701-8.
2. Gonlugur U, Efeoglu T. Vascular adhesion and transendothelial migration of eosinophil leukocytes. *Cell Tissue Res*. 2004 Dec;318(3):473-82.
3. Tai PC, Sun L, Spry CJ. Effects of IL-5, granulocyte/

macrophage colony-stimulating factor (GM-CSF) and IL-3 on the survival of human blood eosinophils in vitro. *Clin Exp Immunol*. 1991 Aug;85(2):312-6.

4. Miotto D, Christodoulopoulos P, Olivenstein R, Taha R, Cameron L, Tsicopoulos A, Tonnel AB, Fahy O, Lafitte JJ, Luster AD, Wallaert B, Mapp CE, Hamid Q. Expression of IFN-gamma-inducible protein; monocyte chemotactic proteins 1, 3, and 4; and eotaxin in TH1- and TH2-mediated lung diseases. *J Allergy Clin Immunol*. 2001 Apr;107(4):664-70.

5. Luster AD. Chemokines--chemotactic cytokines that mediate inflammation. *N Engl J Med*. 1998 Feb 12;338(7):436-45.

6. Platts-Mills TA. The role of immunoglobulin E in allergy and asthma. *Am J Respir Crit Care Med*. 2001 Oct 15;164(8 Pt 2):S1-5.

7. Global Initiative for Asthma. Global strategy for asthma management and prevention: NHLBI/WHO Report 2006; Publication 02-3569.

8. Hochhaus G, Brookman L, Fox H, Johnson C, Matthews J, Ren S, Deniz Y. Pharmacodynamics of omalizumab: implications for optimised dosing strategies and clinical efficacy in the treatment of allergic asthma. *Curr Med Res Opin*. 2003;19(6):491-8.

9. American Thoracic Society/American Lung Association. Recommendations for standardized procedures for the on-line and off-line measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide in adults and children-1999. This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, July 1999. *Am J Respir Crit Care Med*. 1999 Dec;160(6):2104-17.

10. American Thoracic Society. Lung function testing: selection of reference values and interpretative strategies. *Am Rev Respir Dis*. 1991 Nov;144(5):1202-18.

11. Horváth I, Hunt J, Barnes PJ, Alving K, Antczak A, Baraldi E, Becher G, van Beurden WJ, Corradi M, Dekhuijzen R, Dweik RA, Dwyer T, Effros R, Erzurm S, Gaston B, Gessner C, Greening A, Ho LP, Hohlfeld J, Jöbsis Q, Laskowski D, Loukides S, Marlin D, Montuschi P, Olin AC, Redington AE, Reinhold P, van Rensen EL, Rubinstein I, Silkoff P, Toren K, Vass G, Vogelberg C, Wirtz H; ATS/ERS Task Force on Exhaled Breath Condensate. Exhaled breath condensate: methodological recommendations and unresolved questions. *Eur Respir J*. 2005 Sep;26(3):523-48.

12. Prussin C, Metcalfe DD. IgE, mast cells, basophils, and eosinophils. *J Allergy Clin Immunol*. 2003 Feb;111(2 Suppl):S486-94.

13. Adamko DJ, Odemuyiwa SO, Vethanayagam D, Moqbel R. The rise of the phoenix: the expanding role of the eosinophil in health and disease. *Allergy*. 2005 Jan;60(1):13-22.

14. Hamelmann E. The rationale for treating allergic asthma with anti-IgE. *Eur Respir Rev*. 2007 Sept 1; 16(104): 61-6.

15. Humbert M, Beasley R, Ayres J, Slavin R, Hébert J, Bousquet J, Beeh KM, Ramos S, Canonica GW, Hedgcock

S, Fox H, Blogg M, Surrey K. Benefits of omalizumab as add-on therapy in patients with severe persistent asthma who are inadequately controlled despite best available therapy (GINA 2002 step 4 treatment): INNOVATE. *Allergy*. 2005 Mar;60(3):309-16.

16. Djukanović R, Wilson SJ, Kraft M, Jarjour NN, Steel M, Chung KF, Bao W, Fowler-Taylor A, Matthews J, Busse WW, Holgate ST, Fahy JV. Effects of treatment with anti-immunoglobulin E antibody omalizumab on airway inflammation in allergic asthma. *Am J Respir Crit Care Med*. 2004 Sep 15;170(6):583-93.

17. Holgate S, Casale T, Wenzel S, Bousquet J, Deniz Y, Reisner C. The anti-inflammatory effects of omalizumab confirm the central role of IgE in allergic inflammation. *J Allergy Clin Immunol*. 2005 Mar;115(3):459-65.

18. Foroughi S, Foster B, Kim N, Bernardino LB, Scott LM, Hamilton RG, Metcalfe DD, Mannon PJ, Prussin C. Anti-IgE treatment of eosinophil-associated gastrointestinal disorders. *J Allergy Clin Immunol*. 2007 Sep;120(3):594-601.

19. Palframan RT, Collins PD, Williams TJ, Rankin SM. Eotaxin induces a rapid release of eosinophils and their progenitors from the bone marrow. *Blood*. 1998 Apr 1;91(7):2240-8.

20. Woo CH, Jeong DT, Yoon SB, Kim KS, Chung IY, Saeki T, Kim JH. Eotaxin induces migration of RBL-2H3 mast cells via a Rac-ERK-dependent pathway. *Biochem Biophys Res Commun*. 2002 Nov 1;298(3):392-7.

21. Uguccioni M, Mackay CR, Ochensberger B, Loetscher P, Rhee S, LaRosa GJ, Rao P, Ponath PD, Baggiolini M, Dahinden CA. High expression of the chemokine receptor CCR3 in human blood basophils. Role in activation by eotaxin, MCP-4, and other chemokines. *J Clin Invest*. 1997 Sep 1;100(5):1137-43.

22. Miller CW, Krishnaswamy N, Johnston C, Krishnaswamy G. Severe asthma and the omalizumab option. *Clin Mol Allergy*. 2008 May 20;6:4.

23. Zietkowski Z, Skiepkó R, Tomasiak-Lozowska MM, Lenczewska D, Bodzenta-Lukaszyk A. RANTES in exhaled breath condensate of patients with severe persistent allergic asthma during omalizumab therapy. *Int Arch Allergy Immunol*. 2011;154(1):25-32.

24. Zietkowski Z, Skiepkó R, Tomasiak-Lozowska MM, Bodzenta-Lukaszyk A. Anti-IgE therapy with omalizumab decreases endothelin-1 in exhaled breath condensate of patients with severe persistent allergic asthma. *Respiration*. 2010;80(6):534-42.

25. Mochizuki M, Bartels J, Mallet AI, Christophers E, Schröder JM. IL-4 induces eotaxin: a possible mechanism of selective eosinophil recruitment in helminth infection and atopy. *J Immunol*. 1998 Jan 1;160(1):60-8.

26. Teran LM, Mochizuki M, Bartels J, Valencia EL, Nakajima T, Hirai K, Schröder JM. Th1- and Th2-type cytokines regulate the expression and production of eotaxin and RANTES by human lung fibroblasts. *Am J Respir Cell Mol Biol*. 1999 Apr;20(4):777-86.