

The effect of omalizumab on nasal allergic inflammation

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Background: In sensitized patients, coupling between IgE and FcεRI receptors on mast cells leads to release of proinflammatory mediators and a subsequent influx of inflammatory cells to the affected organ. Omalizumab (Xolair; formerly rhuMAB-E25) binds to circulating IgE, thus preventing induction of the allergic process.

Objective: We investigated the effect of treatment with omalizumab on seasonal allergic rhinitis and related changes in inflammatory cell numbers in nasal biopsy specimens.

Methods: Patients were randomized to treatment with omalizumab or placebo before the pollen season; the treatment was started and continued during season. Symptoms and use of medication were recorded, and blood samples and nasal biopsy specimens were obtained before and during season. Immunocytochemistry was performed on biopsy sections through use of the following antibodies: anti-CD4, CD8 (T lymphocytes), EG2, and anti-eosinophil peroxidase (eosinophils), anti-tryptase (mast cells), human neutrophil lipocalin (neutrophils), and antibodies against IgE and FcεRI.

Results: During the season, blood eosinophils increased in placebo-treated patients but not in omalizumab-treated patients ($P = .01$); the difference between the treatment groups was significant ($P = .04$). Free IgE in serum decreased significantly ($P = .0002$) in omalizumab-treated patients but not in placebo-treated patients; the difference between the groups was significant ($P = .0001$). In nasal biopsy specimens, the number of eosinophil peroxidase–positive staining cells increased in the placebo-treated patients ($P = .003$) but not in the actively treated patients during the season; the difference between the groups was significant ($P = .0001$). The number of IgE⁺ staining cells decreased significantly in the omalizumab group during the season in comparison with the placebo group ($P = .04$).

Conclusion: The clinical benefit of treatment with omalizumab is associated with an anti-inflammatory effect on cellular

markers in blood and nasal tissue. (*J Allergy Clin Immunol* 2002;110:68-71.)

Key words: Allergic rhinitis, nasal biopsy, IgE, anti-IgE, eosinophils, omalizumab

Seasonal allergic rhinitis (SAR) is associated with inflammation of the nasal mucosa, characterized primarily by increases in eosinophils and mast cells.¹ IgE plays an important role in the induction of allergic inflammation and can also be produced locally in the nasal mucosa in patients with allergic rhinitis.² Omalizumab (Xolair; formerly rhuMAB-E25), a recombinant humanized monoclonal anti-IgE antibody, is the first anti-IgE agent with clinical benefits in the treatment of SAR³ and allergic asthma.⁴ In induced sputum of allergic asthmatics, pretreatment with omalizumab decreased eosinophil numbers before and after allergen challenge.⁵ The present study was designed to examine the effects of omalizumab on nasal allergic inflammation in patients with SAR.

For the nasal biopsy study, 30 adult patients were recruited at 2 centers participating in a multicenter, placebo-controlled study of the effects of omalizumab on birch pollen–induced SAR.³ All subjects were allergic to birch pollen, as demonstrated by positive skin prick test results and in vitro IgE analysis, and each had a ≥ 2 -year history of birch pollen–induced SAR symptoms. Short-acting antihistamine acrivastine (Semprex) was provided as rescue medication.

According to serum total IgE levels measured at baseline (week 0), patients received either 300 mg of omalizumab (administered subcutaneously) or matching placebo at week 0 and week 4 (serum IgE, ≤ 150 IU/mL) or at weeks 0, 3, and 6 (serum IgE, >150 IU/mL). The first dose was given at or before the start of the birch pollen season. Patients were randomized to active or placebo treatment at a 2:1 ratio.

The patients recorded daily nasal (sneezing and itchy, runny, and stuffy nose) and ocular symptoms (itchy, watery, and red eyes) on a 4-point scale. The results were added; the average constituted the total severity score.

METHODS

Blood samples were obtained for eosinophil counts and measurement of serum IgE levels before and during the pollen season. Nasal mucosa specimens were obtained in randomized order from either the

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Abbreviations used

EPO: Eosinophil peroxidase
FcεRI: High-affinity IgE receptor
SAR: Seasonal allergic rhinitis

right or the left inferior turbinate twice during the study: outside the pollen season (before trial treatment) and at the peak of the season (during trial treatment). Local anesthesia of the inferior nasal turbinate was achieved by means of topical application of 1 mL of a solution of 4% cocaine and 0.02% adrenaline. After 5 minutes, a sub-mucosal injection of 0.5 to 1.0 mL of Xylocain 10 mg/mL with adrenaline 5 µg/mL (Astra, Södertälje, Sweden) was given, and a biopsy specimen was obtained from the medial part of the inferior turbinate through use of Gerritsma forceps (Enter- Woerden, Holland). The biopsy specimen was immediately embedded in Tragacanth gum (Acros Organics NV, Fair Lawn, NJ) and snap-frozen in isopentane cooled with liquid nitrogen. Biopsy specimens were stored at -70°C .

Immunohistology

Immunohistochemical staining with all antibodies except anti-IgE was performed through use of a modified alkaline phosphatase anti-alkaline phosphatase method, as previously described.⁶ For staining, 6 µm-thick sections were cut by cryostat, placed on a slide, and air-dried at room temperature overnight. Slides not used immediately were wrapped in aluminum foil and kept at -70°C . Monoclonal mouse antihuman antibodies were used to stain T lymphocytes (CD4, 1:50; CD8, 1:200) and mast cells (anti-tryptase AA1, 1:100) (all from Dakopatts, Glostrup, Denmark), neutrophils (anti-human neutrophil lipocalin, 1:100; a gift from Dr Christer Pettersson, Pharmacia, Uppsala, Sweden), and high-affinity IgE receptor (anti-FcεRI, 1:100; Upstate Biotechnology, Lake Placid, NY).

To recognize different eosinophil granule proteins, EG2 (anti-eosinophil cationic protein, 1:50) and EPO (anti-eosinophil peroxidase [EPO], 1:400 [a gift of Dr Christer Pettersson, Pharmacia, Uppsala, Sweden]) were used.

The avidin-biotin peroxidase complex technique⁷ was used to visualize sections stained with rabbit polyclonal antibody anti-IgE (1:1000; Dakopatts). Staining was performed in a semiautomated immunostainer (Shandon Cadenza, Astmoor, United Kingdom) programmed for incubations with anti-IgE antibody, biotinylated swine antirabbit antibody (1:400; Dakopatts), and ABCComplex/HRP (Dakopatts). The reaction was visualized through use of 3-amino-9-ethylcarbazole as a substrate (Dako Corporation, Carpinteria, Calif). Sections were counterstained with Mayer's hematoxylin and mounted in aqueous mounting medium.

Human tonsil and polyp tissue obtained at routine operations were included in each staining as positive controls. Mouse IgG1 (Dakopatts) was used as a negative control.

Quantification

Using a Nikon Eclipse E600 microscope at $\times 400$ magnification, 2 readers independently examined coded sections. Two sections of each biopsy were counted. In each section, the number of positive cells was counted through use of the imaging program package Easy Image 2000 Measuring System (Bergström Inst AB, Solna, Sweden). All accessible fields under basal membrane covered by epithelium and in epithelium were counted, and the mean was calculated as the number of cells per square millimeter.

Statistics

In each group, the average symptom score during the season was compared with preseasonal values through use of the Wilcoxon matched pairs (signed-rank) test. For comparison between the treatment groups, a Mann-Whitney *U* test was performed.

Within each treatment group, the numbers of cells expressing each of the markers at baseline and during the pollen season were compared through use of the Wilcoxon matched pairs (signed-rank) test. Comparisons between treatment groups before and during the season were performed through use of the Mann-Whitney *U* test. All correlations were analyzed through use of the Spearman rank correlation test.

Statistica software (StatSoft, Tulsa, Okla) was used. A *P* value of $<.05$ was considered statistically significant.

RESULTS

Clinical symptoms

Thirty patients from 2 centers participating in a multicenter Scandinavian trial were included in this nasal biopsy study. Nineteen patients (9 male and 10 female) were randomized to the omalizumab treatment; 11 patients (5 male and 6 female) were randomized to the placebo group. The patients ranged in age from 20 to 48 years (mean, 31.6 years).

In both treatment groups, significant seasonal increases in all symptoms variables were noted. In addition, use of rescue medication increased significantly in all patients. In this small number of patients, the differences between the active treatment group and the placebo group were not significant.

Blood eosinophils

The total eosinophil number in peripheral blood increased significantly in the placebo group (from 0.22 to 0.28×10^9 per liter; $P = .01$) and remained close to pre-season values in the omalizumab group (0.19 to 0.18×10^9 per liter). The between-group difference in the changes from pre-season to during the season was significant ($P = .04$).

Serum IgE

The levels of IgE were similar before the season and at the start of treatment in the 2 groups. Free IgE levels during the season decreased significantly in the omalizumab-treated patients ($P = .0002$) but remained unaltered in the placebo-treated patients. During the season, the difference between the treatment groups was significant ($P = .0001$).

Nasal biopsy specimens

Table I shows the numbers of cells staining positively for the inflammatory markers chosen. In the placebo group, the numbers of cells positive for each inflammatory marker tended to increase from baseline during the season. In the active treatment group, values for all markers were essentially unchanged. The number of EPO⁺ staining cells increased significantly during the season in comparison with the pre-season in nasal biopsy specimens of the placebo group ($P = .003$), but not in those of the omalizumab-treated group. The change in the eosinophil marker EPO during the season in the active treatment group was significantly different from the corresponding change in the placebo group ($P = .0001$; Fig 1).

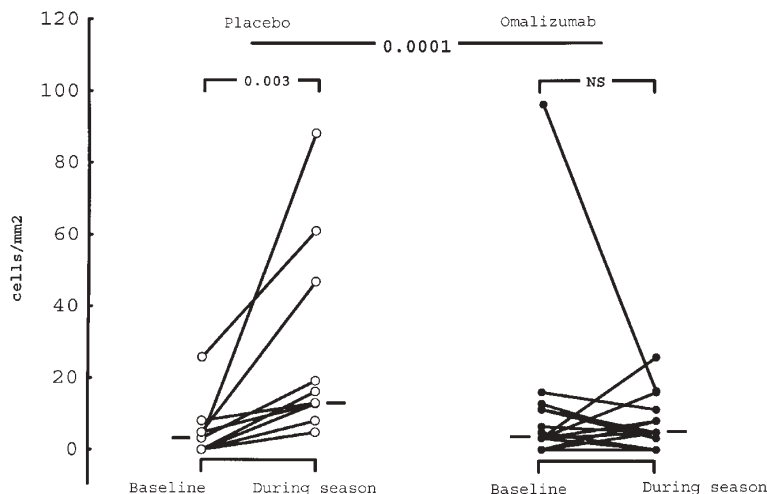


FIG 1. EPO⁺ eosinophil counts (cells/mm²) in nasal biopsy specimens taken before (baseline) and during the birch pollen season in active treatment and placebo groups. Each symbol shows an individual value; horizontal bars are group median values.

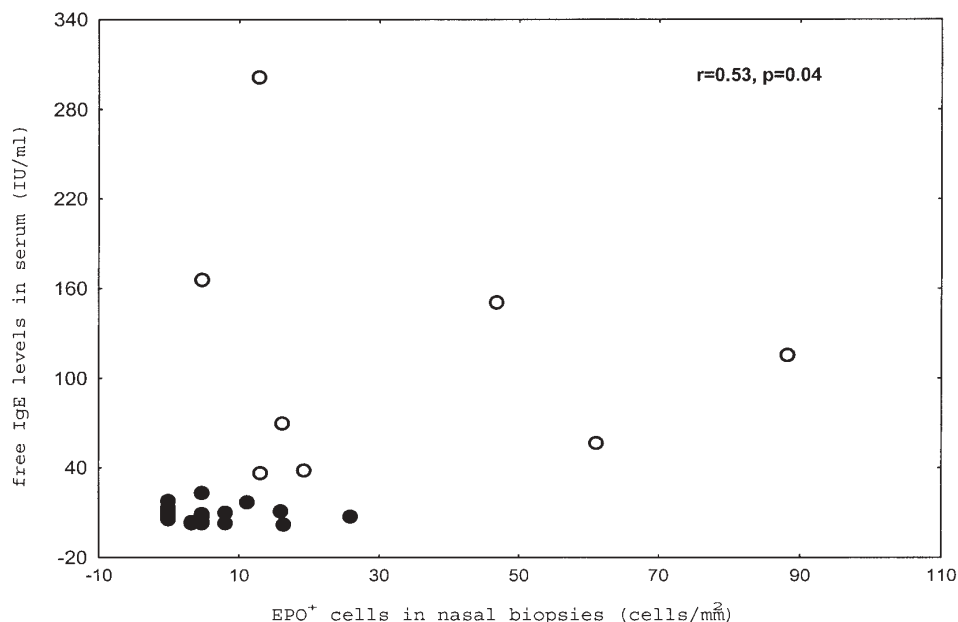


FIG 2. Correlation between serum IgE levels and eosinophils in nasal mucosa during season (open circles, placebo-treated patients; closed circles, actively treated patients).

We found significant correlation between free IgE levels in serum and EPO⁺ cell numbers in nasal biopsy specimens of all patients during the season ($r = 0.39$; $P = .04$; Fig 2). We also found a correlation between free IgE levels in serum and numbers of FcεRI cells in nasal biopsy specimens ($r = 0.40$; $P = .03$) before the season. Correlation between serum IgE and blood eosinophil numbers ($r = 0.43$; $P = .02$) was also significant.

The only significant correlation with clinical variables was found with EG2⁺ cells in nasal biopsy specimens. Thus, the seasonal increase in EG2⁺ cell numbers correlated with nasal symptom severity score ($r = 0.69$; $P = .02$), ocular symptom severity score ($r = 0.61$; $P = .05$),

and total symptom severity score ($r = 0.74$; $P = .01$) in the placebo group but not in the omalizumab-treated group.

DISCUSSION

The results confirm that in birch-sensitized patients, seasonal pollen exposure is associated with inflammation of the nasal tissues, notably in markers reflecting eosinophil activity.¹ Omalizumab appeared to exert an anti-inflammatory effect in preventing allergen-induced tissue eosinophilia, with a corresponding effect on blood eosinophilia.

The results of a statistical analysis performed on the clinical symptoms of the 30 participating patients did not

TABLE I. Number of cells positive for inflammatory markers before (baseline) and during the birch pollen season

Marker	Omalizumab (n = 19)		Placebo (n = 11)		P value (between-group comparison of changes)
	Baseline	During season	Baseline	During season	
CD4 ⁺	65.8 (36.8-114.2)	57.8 (12.8-94.6)	44.8 (20.8-81.4)	53.6 (16.8-79.8)	1.0
CD8 ⁺	129.0 (61.6-161.0)	119.0 (87.0-187.8)	93.2 (49.8-168.8)	158.8 (70.8-200.2)	.9
AA ⁺	39.2 (28.8-67.2)	35.2 (24.0-44.8)	27.2 (19.8-59.2)	36.8 (12.8-82.0)	.4
EG2 ⁺	4.8 (0-16.0)	11.2 (0-29.2)	4.8 (0-31.0)	22.4 (12.8-32.0)	.07
				<i>P</i> = .06 vs baseline	
EPO ⁺	3.2 (0-11.2)	4.8 (0-8.0)	3.2 (0-4.8)	13.0 (8.0-46.8)	.0001
		<i>P</i> = .002 vs placebo		<i>P</i> = .003 vs baseline	
HNL ⁺	38.6 (19.6-48.8)	21.4 (12.8-48.2)	23.6 (14.8-48.2)	16.2 (6.8-30.2)	.8
IgE ⁺	30.6 (10.0-61.3)	18.4 (6.7-70.1)	57.3 (27.1-69.1)	78.7 (28.4-111.8)	.6
		<i>P</i> = .04 vs placebo			
FcεRI ⁺	16.7 (3.3-56.8)	22.4 (6.7-68.5)	30.1 (13.4-77.2)	41.7 (8.4-56.8)	.3

Data are given as numbers of positive cells per square millimeter [median and (interquartile range)].

agree with the findings of the original multicenter study. The explanation lies in too low a number of patients (low power) required to achieve a significant difference between the treated group and the placebo group during the season. The number of patients was, however, sufficient for us to find differences in laboratory variables, such as total eosinophil numbers and levels of free IgE in serum. Furthermore, in nasal biopsy specimens changes in eosinophil numbers turned out to be a sensitive measure of seasonal inflammation.

We used 2 eosinophil specific markers, EPO and EG2, for staining eosinophil granulocytes in the nasal biopsy specimens. EG2 is considered a marker for activated or secreting cells, whereas EPO is sensitive marker for all eosinophils. EPO has been reported to be a more sensitive indicator of atopy and allergic inflammation than EG2.^{8,9} On the other hand, EG2 correlates better with clinical variables, reflecting possibly different kinetics of EG2 production during the season.¹⁰ However, the tendency of seasonal changes for both eosinophil markers in tissue is the same and is in concordance with the results obtained from eosinophil counts in peripheral blood.

By binding to free IgE, omalizumab prevents the interaction between IgE and its high-affinity FcεRI receptors on mast cells and basophils, so that IgE cross linking and cell activation cannot occur.¹¹ The reduction in free IgE is accompanied by a reduction in FcεRI expression on circulating basophils and thus in cell responsiveness to allergen stimulation.¹² However, the relationship between serum IgE and FcεRI receptor occupancy on tissue cells is not clear. We have observed correlation between free IgE in serum and numbers of FcεRI⁺ cells in nasal tissue outside the pollen season.

The significant reduction in EPO⁺ cells and the correlation with serum IgE that we observed support the role of IgE in mediating eosinophil cytotoxicity,¹³ and the overall profile of effects of anti-IgE on eosinophilic variables underlines the important role of IgE in allergic inflammation.

In the original multicenter study, omalizumab controlled birch pollen-induced SAR symptoms with less concomitant medication use and improved rhinoconjunctivitis-specific quality of life in comparison with placebo.³

Those results and the results obtained in the present study suggest that omalizumab has clinical benefits in SAR that are accompanied by favorable effects on allergic inflammatory variables, particularly eosinophils in peripheral blood and affected tissue.

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