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To cite this article: Weronika Zastrzeżyńska, Marek Przybyszowski, Stanisława Bazan-Socha, Agnieszka Gawlewicz-Mroczka, Piotr Sadowski, Krzysztof Okoń, Bogdan Jakiela, Hanna Plutecka, Adam Ćmiel, Krzysztof Śladek, Jacek Musiał & Jerzy Soja (2019): Omalizumab may decrease the thickness of the reticular basement membrane and fibronectin deposit in the bronchial mucosa of severe allergic asthmatics, Journal of Asthma, DOI: [10.1080/02770903.2019.1585872](https://doi.org/10.1080/02770903.2019.1585872)

To link to this article: <https://doi.org/10.1080/02770903.2019.1585872>



Published online: 23 Mar 2019.



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Omalizumab may decrease the thickness of the reticular basement membrane and fibronectin deposit in the bronchial mucosa of severe allergic asthmatics

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ABSTRACT

Introduction: Immunoglobulin E is an important modulator of the inflammatory reaction in allergic asthma. It also contributes to airway remodeling in the course of the disease. The authors evaluated airway structural changes in severe allergic asthma during the omalizumab therapy. **Patients and methods:** The study included 13 patients with severe allergic asthma treated with omalizumab for at least one year. In each patient clinical, laboratory, and spirometry parameters were evaluated before and after the treatment. In addition, bronchoscopy with bronchial mucosa biopsy and bronchoalveolar lavage was performed. The basal lamina thickness, inflammatory cell infiltration, fibronectin, as well as type I and III collagen accumulation were assessed in bronchial mucosa specimens, together with the assessment of bronchoalveolar lavage cellularity. **Results:** The omalizumab therapy led to a decrease in the basal lamina thickness ($p=0.002$), and to a reduction in fibronectin ($p=0.02$), but not collagen deposits in the bronchial mucosa. The decrease in fibronectin accumulation was associated with an improvement in asthma control and quality of life ($p=0.01$, both), and a diminished dose of systemic corticosteroids ($p=0.001$). It was also associated with a tendency towards reduction of the eosinophil count in the peripheral blood, bronchoalveolar lavage fluid, and bronchial mucosa specimens. **Conclusion:** Our study has shown that omalizumab, effective in the treatment of severe allergic asthma, may also decrease unfavorable structural airway changes in allergic asthmatics, at least with respect to the fibronectin deposit and an increased thickness of the basal lamina. However, more extensive observational studies are needed to verify the above hypothesis.

ARTICLE HISTORY

Received 30 November 2018
Revised 16 February 2019
Accepted 18 February 2019

KEYWORDS

Asthma; omalizumab;
airway remodeling;
fibronectin; IgE

Introduction

Asthma is a chronic inflammatory disease of the airways [1]. Persistent inflammation, particularly in severe asthma, may lead to structural changes in the bronchial wall, termed remodeling [1]. Such changes occur in response to chronic inflammation and refer to an increased thickening of the bronchial wall [2]. Among pathological features characteristic of airway remodeling, the most important are hypertrophy of the mucous glands and goblet cells, an increased thickness of the basal membrane, proliferation of the fibroblasts and myofibroblasts in the submucosa, as well as airway smooth muscle hypertrophy and

neovascularization [1–3]. In the pathogenesis of the described structural changes, epithelial damage by various inflammatory mediators is postulated as a triggering factor [1,2]. Dysfunction of the airway epithelial cells leads to the impairment of their protective function and an increased susceptibility to harmful factors, including irritants or allergen components [1–3]. On the other hand, selected inflammatory cytokines, such as the transforming growth factor β , stimulate the synthesis of procollagens and other matrix proteins, leading to the excessive extracellular deposition of collagens I (III) and fibronectin within the basal membrane. Additionally, as it has been

demonstrated, the inflammatory cytokines, such as tumor necrosis factor α , interleukin (IL)-1 β , IL-5, and IL-13, stimulate proliferation of the fibroblasts, goblet cells, and smooth muscle cells in the bronchial wall. Moreover, an increased number of the myocytes demonstrate excessive contractility and evoke irreversible obstruction. They are also a source of other proinflammatory mediators and promote angiogenesis, another important feature of airway remodeling. In turn, pathological neovascularization is related to congestion and an increased permeability, leading to the bronchial wall edema and a further decrease in bronchial lumen [4–6]. Currently, there is no effective cure of asthma airway remodeling. In recent years, several reports have indicated a significant role of immunoglobulin (Ig)E and its receptors in the development of this condition [7,8]. The high-affinity IgE receptor is present on the infiltrating inflammatory cells, but also on the airway epithelial and smooth muscle cells [8,9]. Roth et al. [10] in their experimental study have demonstrated that stimulation of the bronchial smooth muscle by IgE leads to the overproduction of type I, III, and VII collagens and fibronectin, important components of the extracellular matrix (ECM). Interestingly, the pre-incubation of these cells with omalizumab results in a complete inhibition of the above activity [11]. It has been also previously shown that omalizumab diminishes the expression of high affinity IgE receptors on the mast cells, basophiles [12], and dendritic cells [13]; thus, it might inhibit the IgE-dependent activation and release of proinflammatory mediators by these cells [14]. Moreover, the anti-IgE therapy has been demonstrated to be related to the reduction of dendritic cell and eosinophil numbers in bronchial biopsies [15–17], as well as of eosinophil count in the peripheral blood and sputum of asthmatic subjects [16]. Due to this property, omalizumab might affect airway remodeling, since eosinophils play an important role in the above condition [18].

In the present study we have sought to investigate whether the regular treatment of severe allergic asthma with omalizumab might decrease unfavorable structural changes of airway remodeling in parallel with the clinical improvement and reduced inflammatory cells infiltration.

Patients and methods

Patients

The study included 13 patients with severe allergic asthma treated with omalizumab for at least 12 months. Severe asthma was defined according to the GINA guidelines [19,20] as a severe persistent disease despite the treatment with a high daily dose of

inhaled corticosteroids (ICS) ($>500 \mu\text{g}$ of fluticasone propionate [dry powder inhaler] or its equivalent). At the time of recruitment to the study, all the patients also received long-acting β_2 agonists and oral corticosteroids. The dosage and frequency of omalizumab administration was based on the IgE concentration measured before the initiation of the treatment, and on the patient's body weight, according to the reference recommendation. Spirometry was performed using a Jaeger, Master Screen spirometer in reference to the American Thoracic Society standards.

The study was approved by the Ethics Committee of the Jagiellonian University. All the subjects gave written informed consent to participate in the study.

Bronchofiberoscopy

Bronchofiberoscopy with collection of bronchoalveolar lavage fluid (BAL) and bronchial biopsy were performed two times in each patient, according to the guidelines of the American Thoracic Society [19] using a BF 1T180 bronchofibroscope (Olympus, USA), with the patient under local anesthesia (2% lidocaine) and under mild sedation (0.05–0.1 mg fentanyl and 2.5–5 mg midazolam, both given intravenously). During that procedure, BAL was carried out with 200 ml of 0.9% saline administered to the right middle lobe bronchus, and 2–3 bronchial biopsy specimens were collected from the right segmental basal bronchi (the carina between B9 and B10) for further histological investigation. The first bronchofiberoscopy was performed up to two weeks before the first dose of omalizumab, while the second after at least 12 months of the regular omalizumab therapy. The period between the first and the second bronchofiberoscopy differed among the patients, with the median value of 22 months (range: 13–30 months). The second bronchoscopy was an additional procedure, not necessary for the continuation of the omalizumab treatment. Therefore, some patients did not agree to the second procedure to be performed exactly after 12 months of the treatment and asked for a longer time for consideration. However, what is important, the biological therapy was always continued during the period up to the second bronchofiberoscopy.

Histology and immunohistochemistry

The tissue was fixed in 10% buffered formalin, routinely processed and embedded in paraffin. The $2 \mu\text{m}$ sections were prepared and stained by the hematoxylin-eosin method. The images were recorded using an Axioscop microscope (Zeiss, Germany) equipped with

a 100× oil-immersion lens and a Nikon D5100 digital camera. In each case, all the images containing identifiable basement membrane were recorded. The thickness of the basement membrane was measured with an imaging system (AnalySIS imaging system; Soft Imaging System GmbH; Germany) and a custom-built application written in the Imaging C language. The images were acquired all along the available basement membrane, and the thickness was measured with an arbitrary-distance software tool and recorded in a text file. For each patient, at least 30 individual measurements were performed. In each case, the arithmetic and harmonic means were calculated using the Excel spreadsheet (Microsoft Corp., USA).

Immunohistochemistry for the CD3+ and CD20+ cells was performed according to the protocol routinely used in our laboratory. The selected blocks were cut into 4 µm-thick sections. Antigen retrieval was performed by incubating the slides in citrate buffer (pH 6.0; 0.01 mol/l) at 97°C in a water bath for 40 min. The employed primary antibodies were as follows: for CD20 – monoclonal mouse antibody clone L26 (Dako, Denmark), for CD3 – monoclonal mouse antibody clone F7.2.38 (Dako, Denmark). The UltraVision Quanto detection system (Lab Vision, ThermoScientific, USA) and 3,3'-diaminobenzidine as chromogen were applied, and the slides were counterstained with Mayer hematoxylin (Thermo Fisher Scientific, Waltham, USA) and cover-slipped. The eosinophils were identified by the presence of extensive pinkish granules in their cytoplasm on the hematoxylin-eosin stained slides. In each type of the investigated cells, the eosinophils were counted under a microscope with 400× magnification, in 10 high power fields or, if the biopsy sample was smaller, in the entire section. The number of cells was expressed as the number of cells per 10 microscopic high-power fields.

To evaluate the amount of collagen type I and type III, as well as fibronectin, 2 µm sections were prepared, using the routine laboratory manner as described above. The employed primary antibodies were as follows: for collagen I – monoclonal rabbit antibody clone EPR7785 (Abcam, UK), for collagen IV – monoclonal mouse antibody clone FH-7A (Abcam, UK), for fibronectin – polyclonal rabbit antibody A025 (Dako, Denmark).

The results were assessed semiquantitatively and scored as 0 (lack of staining), 1 (very faint staining), 2 (evident stain), 3 (strong reaction) [strength of the reagent of ECM components = ECM staining level], as well as by estimating the surface area of the section percentage that showed reactivity for the selected antibodies [ECM deposition].

BAL analysis

The cytospin preparations (Thermo Scientific, Waltham, MA) were made from the BAL fluid samples and stained with the May-Grunwald Giemsa dye. One thousand cells in each preparation were counted, the results were shown as the percentage of all the inflammatory cells (with exception of the epithelial cells).

Clinical assessment and basic laboratory tests

Before the initiation of the omalizumab therapy, the serum IgE level was analyzed by latex nephelometry (Siemens, Marburg, Germany) and the patient's atopy status was assessed by the measurement of allergen-specific IgE (CAP system, Siemens, Marburg, Germany) and/or by skin prick testing (Allergopharma, Reinbeck, Germany).

At the baseline and after 52 weeks of the omalizumab treatment, basic laboratory tests (blood cell and platelet count, alanine and aspartate aminotransferases, creatinine, and urea level determinations) were performed employing routine laboratory techniques. Moreover, we collected the spirometry values, results of the Asthma Control Questionnaire (ACQ) and Asthma Quality of Life Questionnaire (AQLQ), as well as analyzed the asthma treatment mode before and after one year of the biological therapy, together with the number of severe asthma exacerbations during the entire period of omalizumab administration. A severe asthma exacerbation was defined according to the American Thoracic Society/European Respiratory Society Statement [21,22], as an episode characterized by a progressive increase in asthma symptoms, which required the use of systemic corticosteroids (tablets, suspension, or injection), or an increase from a stable maintenance dose, at the physician's discretion, for at least 3 days, and/or hospitalization or an emergency visit because of asthma requiring systemic corticosteroids. The effectiveness of the omalizumab therapy was assessed using the Global Evaluation of Treatment Effectiveness [(GETE) scale: 1 = excellent, 2 = good, 3 = moderate, 4 = poor, 5 = worsening]. All the enrolled patients demonstrated at least a good clinical response to the treatment.

Statistical analysis

The results were compared using the Dell Statistica (data analysis software system), version 13. software.-dell.com. Continuous variables were reported as medians and quartiles. Categorical variables were

reported as percentages. The Box-Cox normality transformations were used when needed. Normality was checked using the Shapiro-Wilk *W*-test. The Student *t*-test for normally distributed (after Box-Cox transformation) samples or the Wilcoxon signed rank test for non-normal samples were used for comparing two dependent samples. The general linear model (GLM) was also used for taking into account additional factors in intergroup comparisons. The Spearman rank correlation coefficients were used to analyze the associations between two selected parameters. The results were considered significant when the *p* values were less than 0.05.

Results

Patients

Eleven patients of 13 subjects treated with omalizumab (84.6%) were females. The median age at the asthma diagnosis was 22 years (range: 4–33), while the median age at the onset of the omalizumab treatment was 48 years (range: 39–56). The median IgE level was 351 IU/ml (range: 203–505). Clinical and laboratory characteristics of the asthma patients at the baseline and one year after the omalizumab therapy are presented in Table 1. As it was demonstrated, the asthmatics reported low quality of life (AQLQ < 5) and lack of asthma control (ACQ > 1.5) before the omalizumab treatment. All the patients included in the study experienced at least three asthma exacerbations in the year preceding the omalizumab therapy, while in six subjects (46.2%), the exacerbations required hospitalization. Moreover, two (15.4%) asthmatics had life-threatening asthma attacks in the past, and four (30.8%) subjects presented with irreversible severe bronchial obstruction with forced expiratory volume in 1 s (FEV₁) lower than 60% of the predicted value.

Clinical effectiveness of omalizumab

As shown in Table 1, the omalizumab therapy was related to a significant reduction in the systemic corticosteroid dose ($p < 0.001$), improvement in the AQLQ and ACQ score (of 1.3 and 0.8 point, respectively; $p < 0.001$, both), as well as a 15% increase in FEV₁ ($p = 0.025$). We also documented a decreased exacerbation rate after the biological treatment ($p < 0.001$) (Table 1).

Immunochemistry

At the baseline, the fibronectin staining level in the mucous membrane continued to be positively associated with the ACQ score ($R = 0.56$, $p = 0.03$). On the other hand, of all the cells infiltrating the bronchial mucosa, the only correlation was documented between the fibronectin staining level and the number of T cells (an inverse relationship – $R = -0.54$, $p = 0.04$).

As mentioned earlier, during the omalizumab therapy, we observed a decrease in the number of exacerbations compared to the period before the treatment. Individually, four patients had no asthma exacerbations, four had one and five patients had at least two asthma exacerbations. After the therapy, the individuals who experienced more than one exacerbation (Group 1 represented the patients who manifested one exacerbation episode at the maximum during the treatment with omalizumab, $n = 8$) compared to Group 2 (the patients demonstrated a minimum of two exacerbations, $n = 5$) demonstrated a thicker basement membrane, but the difference was not statistically significant.

Interestingly, in the subjects with at least two exacerbations during the omalizumab therapy ($n = 5$, 38%), the fibronectin staining level in the mucous membrane at the baseline also continued to show a positive association with oral corticosteroid dose ($R = 0.894$, $p = 0.041$) and fibronectin deposition had

Table 1. Clinical and laboratory characteristics of asthma patients at the baseline and after one year of the omalizumab therapy. The data are presented as median and interquartile range.

	Baseline <i>n</i> = 13	52 weeks of treatment <i>n</i> = 13	<i>p</i>
Inhaled corticosteroid dose, µg recalculated to fluticasone propionate	2000 (1000–2750)	2000 (1000–3500)	$p = 0.449$
Oral corticosteroid dose, mg recalculated to methylprednisolone	8 (4–15)	1 (0–8)	$p < 0.001$
Asthma Control Questionnaire, number of points	2.9 (2.8–3.9)	2.10 (1.14–2.30)	$p < 0.001$
Asthma Quality of Life Questionnaire, number of points	3.8 (3.0–4.0)	5.06 (4.30–5.80)	$p < 0.001$
Forced expiratory volume in 1 second, % of predicted value	61 (56–70)	76 (60–79)	$p = 0.025$
Exacerbations, number in the last year	4 (3–6)	0 (0–1)	$p < 0.001$
Blood eosinophilia, number/µl	200 (130–300)	250 (120–370)	$p = 0.261$
Blood monocytes, number/µl	550 (500–760)	520 (430–620)	$p = 0.821$
Alanine aminotransferase (IU/L)	27 (24–44)	28 (23–33)	$p = 0.295$
Aspartate aminotransferase (IU/L)	20 (17–27)	22 (20–28)	$p = 0.927$
Creatinine, µmol/l	67 (60–81)	67 (60–83)	$p = 0.657$
Urea, mmol/l	5.2 (4.1–5.9)	5.45 (4.15–6.40)	$p = 0.337$

a negative association with FEV_1 ($R = -0.975$, $p = 0.005$), both at the baseline. Moreover, we demonstrated that the fibronectin staining level (the strength of the reagent for fibronectin) assessed before the treatment was positively associated with the ACQ score assessed 16 weeks and 1 year after the onset of the treatment ($R = 0.68$, $p = 0.01$ and $R = 0.64$, $p = 0.004$, respectively) and inversely associated with the AQLQ score measured at the same time points ($R = -0.79$, $p = 0.001$ and $R = -0.84$, $p < 0.001$, respectively).

We also documented a positive correlation between the thickness of the basement membrane at the baseline and the number of exacerbations in the year preceding the omalizumab therapy ($R = 0.65$, $p = 0.024$). Moreover, in the pretreatment mucosa specimens, the thickness of the lamina propria was inversely related to the number of the mast cells in biopsy specimens ($R = -0.756$, $p = 0.03$).

No correlation was documented between the basal lamina thickness and the ACQ and AQLQ scores, spirometry values, oral corticosteroid dose, and peripheral blood or BAL eosinophilia prior to the treatment.

As shown in Figures 1 and 2, the omalizumab therapy was associated with a 22% decrease in the basal lamina thickness (6.54 [5.35–7.15] vs. 5.12 [4.9–5.9], $p = 0.002$) and a 33% decline in the fibronectin deposit (75 [90–50] vs. 50 [75–25], $p = 0.024$) in the bronchial mucosa specimens. No such observations were demonstrated either for collagen type I, or type III (Table 2).

Interestingly, we observed a strong positive association between the reduction of fibronectin deposit (shown as the percentage of the stroma showing reactivity after the treatment vs. prior to the therapy) and the reduction of oral corticosteroid dose after the omalizumab therapy ($R = 0.71$, $p = 0.001$, Figure 3). Moreover, the decrease in the level of fibronectin staining (the strength of the reagent for fibronectin, after the treatment vs. prior to the therapy) correlated positively with the AQLQ score assessed one year after the treatment ($R = 0.748$, $p = 0.001$), and negatively with the ACQ score at the same time point ($R = -0.68$, $p = 0.01$).

The omalizumab therapy was also related to the tendency towards reduction of the eosinophil count in all the three compartments: peripheral blood (200 [130–300] vs. 140 [90–300]/ μ l), BAL (1.7 [1–5.1] vs. 1.24 [0.80–2.36]/ μ l), bronchial mucosa sections (0.47 [0.17–0.65] vs. 0.23 [0.00–0.56] the number of cells per 10 microscopic high-power fields), although the

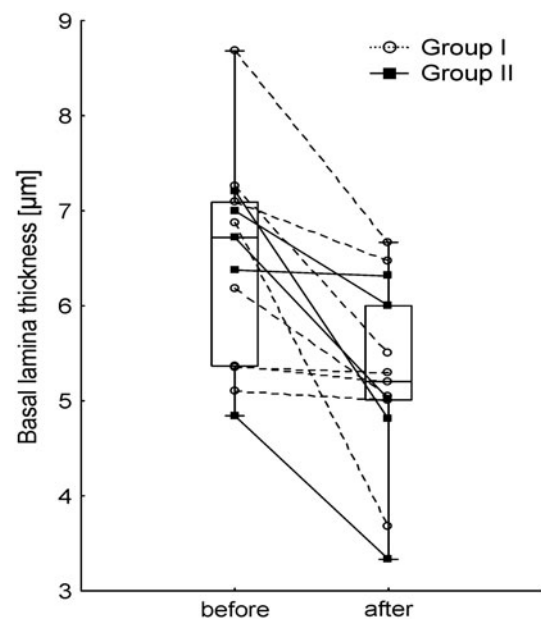


Figure 1. Changes in basal lamina thickness before and after omalizumab treatment (measured in micrometers). Group 1 represented patients who manifested one exacerbation episode at the maximum during treatment with omalizumab, $n = 8$) compared to Group 2 (patients demonstrated a minimum of two exacerbations, $n = 5$). Data is presented as median with upper and lower quartile, and minimum and maximum value.

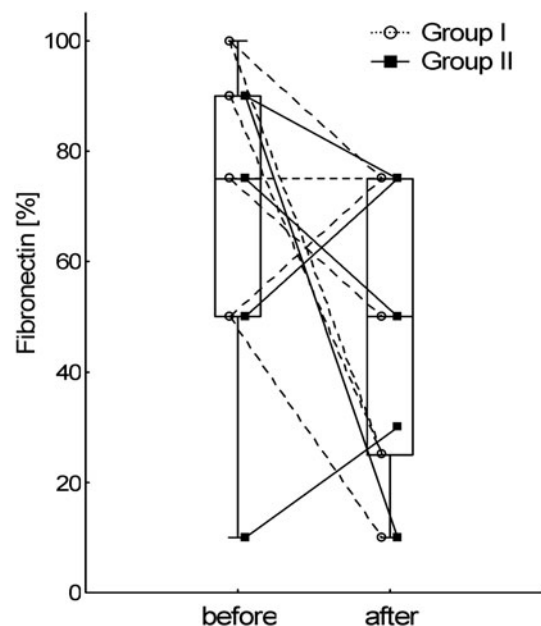


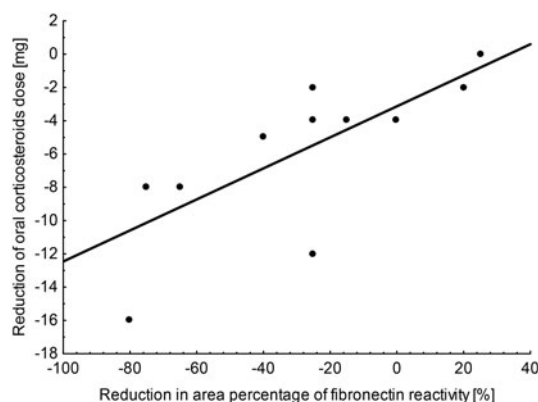
Figure 2. Changes in the fibronectin deposit (area percentage of reactivity) in airway mucosa before and after omalizumab treatment. Group 1 represented patients who manifested one exacerbation episode at the maximum during treatment with omalizumab, $n = 8$) compared to Group 2 (patients demonstrated a minimum of two exacerbations, $n = 5$). Data is presented as median with upper and lower quartile, and minimum and maximum value.

Table 2. Results of the histological study of the bronchial mucosa and cell analysis in bronchoalveolar lavage fluid at the baseline and after the omalizumab therapy.

	At the baseline <i>n</i> = 13	After omalizumab treatment <i>n</i> = 13	<i>p</i>
Collagen type III (ECM staining level)	2 (2–3)	2 (2–3)	<i>p</i> = 0.231
Collagen type III (ECM deposition)	50 (40–75)	65 (50–75)	<i>p</i> = 0.314
Collagen type I (ECM staining level)	2 (1–2)	2 (1–2)	<i>p</i> = 0.953
Collagen type I (ECM deposition)	25 (5–40)	30 (25–50)	<i>p</i> = 0.073
Fibronectin (ECM staining level)	2 (1–2)	2 (1–2)	<i>p</i> = 0.770
Fibronectin (ECM deposition)	75 (50–90)	50 (25–75)	<i>p</i> = 0.024
CD3+ (infiltration into the bronchial mucosa, the number of cells per 10 microscopic high-power fields)	16 (10–27)	20 (16–34)	<i>p</i> = 0.279
CD20+ (infiltration into the bronchial mucosa, the number of cells per 10 microscopic high-power fields)	2 (1–5)	2 (0.5–5)	<i>p</i> = 0.961
Eosinophils (infiltration into the bronchial mucosa, the number of cells per 10 microscopic high-power fields)	0.47 (0.17–0.65)	0.24 (0.00–0.56)	<i>p</i> = 0.436
Macrophages (percentage of all the cells in BAL)	88 (74–92)	80 (79–88)	<i>p</i> = 0.753
Neutrophils (percentage of all the cells in BAL)	2 (0.9–3.6)	1.5 (1–3.2)	<i>p</i> = 0.507
Eosinophils (percentage of all the cells in BAL)	1 (0.5–2.9)	0.5 (0.4–1)	<i>p</i> = 0.247
Mast cells (percentage of all the cells in BAL)	0 (0–0)	0 (0–0.1)	<i>p</i> = 0.345
Lymphocytes (percentage of all the cells in BAL)	7 (4–16)	7 (4–12)	<i>p</i> = 0.727
Macrophages / μ l	104 (49–158)	148 (110–246)	<i>p</i> = 0.066
Neutrophils / μ l	2 (2–6)	3 (1–4)	<i>p</i> = 0.515
Lymphocytes / μ l	10 (7–24)	8 (7–22)	<i>p</i> = 0.757
Eosinophils / μ l	1.7 (1–5)	1.24 (0.8–2.36)	<i>p</i> = 0.505

The data are presented as median and interquartile range.

Abbreviations: ECM: extracellular matrix; BAL: bronchoalveolar lavage.

**Figure 3.** Association of reduction in fibronectin deposit with diminished dose of oral corticosteroids (recalculated to methylprednisolone) after 12 months of omalizumab treatment ($R = 0.71$, $p = 0.001$).

differences were not statistically significant. The infiltration of the other investigated inflammatory cells (CD20+ and CD3+) into the bronchial mucosa was not changed after the omalizumab therapy. Likewise, the BAL composition was similar before and after omalizumab administration (Table 2).

Discussion

Omalizumab has been demonstrated to be clinically effective and almost free from side effects in the

therapy of severe allergic asthmatics [20, 22]; however, its effect on remodeling continues to be virtually not understood.

In the present study, we have shown that the anti-IgE treatment may decrease unfavorable structural airway changes. At least one-year omalizumab administration has led to reduction in the fibronectin deposit and thinning of the increased reticular basement membrane in bronchial mucosa specimens of severe allergic asthmatics. To date, only few reports have addressed the impact of omalizumab on structural airway changes in asthmatics. Hoshino et al. [23] have shown that anti-IgE therapy is related to the decrease in airway wall thickness analyzed in chest computed tomography. Thinning of the increased reticular basement membrane has been also demonstrated in a group of 11 asthmatics treated with omalizumab for one year [24]. Moreover, Mauri et al. [25] have shown that the anti-IgE therapy may decrease smooth muscle proteins in the bronchi, mainly myosins and actins, in the group of patients who responded well to the treatment. Interestingly, in the above study, the protein profile in omalizumab responders was significantly different from those of non-responders before and after the treatment [25]. In turn, it has been shown that the level of galectin-3 at the baseline might be a biomarker of reduction in bronchial reticular basement membrane thickness, eosinophilic inflammation

and muscular and fibrotic components after 3 years of the omalizumab therapy [26].

Currently, we have confirmed that omalizumab leads to the reduction in the bronchial reticular basement membrane thickness and extended the observation adding the data on the diminished fibronectin deposit after the omalizumab therapy. Based on the current report by Riccio et al. [26], we have also divided the patients into two groups, according to their response to the treatment: a very good response or a good response (based on the GETE scale). However, both these groups have not differed in the thickness of the basal membrane, fibronectin level or collagen before or after the treatment (data not shown). Of note, our analysis has not included patients who didn't respond sufficiently to the treatment during the first 16 weeks of omalizumab administration (GETE grade 3–5). In those subjects, the cost of omalizumab has ceased to be reimbursed and the therapy had to be stopped after the first 16 weeks.

The relationship between structural airway changes and inflammation in asthma is currently under discussion. Remodeling is a complex process that may develop as a consequence of persistent inflammation and/or aberrant tissue repair mechanisms. An increased accumulation of fibronectin and collagen in the subepithelial compartment has been observed in all the asthma phenotypes, even at the beginning of the disease [27,28]. Moreover, it has been demonstrated that fibronectin accumulation in the bronchial mucosa leads to further deposition of other ECM proteins, including collagens, tenascin, and vitronectin [29]. These unfavorable abnormalities might be related to modification in the lung stroma and in the mechanical properties of the airways [27–30]. Airway structural alterations and inflammation in patients with asthma are believed to play a role in disease severity. Patients with severe asthma have thicker airway walls as measured by computed tomography scans when compared to patients with mild asthma or to healthy subjects [31]. Moreover, their bronchial biopsy specimens show an increased vessel area [32] and airway smooth muscle [6], as well as thickened bronchial epithelium [33]. Furthermore, in the alveolar parenchyma, patients with uncontrolled asthma demonstrate an increased percentage of areas of collagen, versican, and decorin, together with significantly higher numbers of myofibroblasts in both the central airways and alveolar parenchyma as compared to patients with well-controlled asthma [34].

In the present study, we have demonstrated for the first time that fibronectin deposits decrease after the

omalizumab therapy. Moreover, the said reduction is associated with an improvement in clinical asthma parameters. Taking into account the above observation and the fact that a higher fibronectin level at the baseline is related to worse asthma control, lower FEV₁, and an increased dose of oral corticosteroids at enrollment in subjects with a higher exacerbation rate on omalizumab, one might speculate that a higher fibronectin content in the lamina propria indicates a worse asthma course. This hypothesis, however, requires further investigation.

The positive correlation between a decrease in fibronectin deposit and the reduction in the dose of systemic glucocorticosteroids, as well as an improvement in asthma control and quality of life after the omalizumab treatment deserves a comment. Ferreira et al. [35] have demonstrated that the severity of airway inflammation and remodeling changes are not related to the asthma symptoms. However, other studies do not support this hypothesis [27, 30]. Our data have also demonstrated that the reduction in fibronectin deposit in the airway mucosa might be a determinant of asthma improvement, at least during the omalizumab therapy.

Interestingly, the reduction of fibronectin content in our study has not been related to the diminished collagen deposits which are also important in asthma pathology [36]. We do not know what the explanation of this discrepancy is; however, it might be possible that collagen accumulation is not reversible or requires a longer duration of the therapy. This issue will be addressed in our future research.

In our study, the basal lamina thickness at enrollment has remained in a positive association with the number of exacerbations prior to the treatment, indicating that an increased exacerbation rate may lead to the unfavorable airway structural changes in asthmatics. However, a negative correlation between the lamina propria thickness and the number of mast cells in the pretreatment bronchial mucosa specimens has been a surprising finding. The observation is contrary to other reports demonstrating that more severe asthma is related to a higher number of mast cells in the lung tissue [37,38]. On the other hand, a study has been recently published showing that asthmatics with persistent airflow obstruction have a thicker layer of the airway smooth muscle, in addition to lower numbers of the mast cells in the submucosa as compared to subjects with non-persistent obstruction [35], what is in accordance with our report.

Eosinophils are other important cells in allergic inflammation, associated with tissue damage and

remodeling [39]. Sputum eosinophilia correlates with asthma severity and is associated with a higher risk of severe exacerbation [40,41]. Eosinophils may also contribute to tissue remodeling by regulating the deposition of ECM proteins. Mepolizumab, an anti-IL-5 antibody, has decreased the blood and airway eosinophil count, as well as reduced the expression of tenascin, lumican, and procollagen III in the bronchial mucosa lamina propria [42].

Omalizumab may also reduce lung and blood eosinophilia [39], what also likely contributes to the decreased airway inflammation and remodeling.

Study limitations

Obviously, our study has several limitations. First, the analyzed group is relatively small. We have not assessed other histological features of airway remodeling, including the smooth muscle layer or neovascularization. We have also not studied the deposits of other ECM components in the bronchial mucosa, e.g. laminin or tenascin. Those traits, however, are beyond the scope of our study. We have not been able to exclude focal rather than continuous changes in the lamina propria, particularly in view of the fact that we have examined specimens originating from one location only. The period between the first and the second bronchoscopy has differed among patients. However, the second bronchoscopy was performed always during omalizumab therapy. Finally, the clinical relevance of the demonstrated structural airway changes, as well as their impact on disease severity, progression, and the relation to the functional abnormalities require further investigations.

Conclusions

Our study has shown that omalizumab, effective in treatment of severe allergic asthma, may also decrease unfavorable airway structural changes in allergic asthmatics, at least with respect to the fibronectin deposit and increased thickness of the basal lamina. However, our suggestions need to be verified by larger observational studies.

Acknowledgements

We wish to thank various people for their contribution to this project: Anna Andrychiewicz- coordinating nurse in the Bronchoscopy Laboratory, for valuable technical support on this project, and Anna Rams for help in data collection.

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Funding

This project was funded by the National Science Centre based on decision No: DEC-2013/09/B/NZ5/00758 (to S. B-S.).

Authors' contribution

Weronika Zastrzeżyńska, Krzysztof Śladek, and Jerzy Soja contributed Study Design.

Weronika Zastrzeżyńska, Marek Przybyszowski, Stanisława Bazan-Socha, Agnieszka Gawlewicz-Mrocza, and Jerzy Soja contributed Data Collection.

Weronika Zastrzeżyńska, Stanisława Bazan-Socha, Adam Ćmiel, and Jerzy Soja contributed Statistical Analysis.

Weronika Zastrzeżyńska, Stanisława Bazan-Socha, Krzysztof Śladek, and Jerzy Soja contributed Data Interpretation.

Weronika Zastrzeżyńska, Stanisława Bazan-Socha, Krzysztof Śladek, Jacek Musiał, and Jerzy Soja Manuscript Preparation.

Weronika Zastrzeżyńska, Stanisława Bazan-Socha, and Jerzy Soja contributed Literature Search.

Krzysztof Śladek and Jerzy Soja contributed Bronchofiberscopy.

Krzysztof Okoń contributed Histology and immunohistochemistry.

Piotr Sadowski and Krzysztof Okoń contributed Cells count.

Bogdan Jakiela and Hanna Plutecka contributed BAL analysis.

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