

Original article

Omalizumab decreases nonspecific airway hyperresponsiveness *in vitro*

Background: In asthmatic patients, both symptoms and hyperresponsiveness are related to immunoglobulin E (IgE) concentration in serum. The anti-IgE monoclonal antibody omalizumab improved the control of asthma, but its effect on airway hyperresponsiveness is controversial. Passive sensitization reproduced *in vitro* a bronchial hyperresponsiveness, an increase in IgE bearing cells, and a mast cell degranulation. This study was designed to examine the effect of omalizumab on passive sensitization-induced hyperresponsiveness, alterations in IgE positive inflammatory cells and mast cell degranulation within the bronchial wall.

Methods: Proximal (3–5 mm diameter) and distal (0.5–1.5 mm diameter) human bronchi dissected out from 10 lung specimens were incubated in normal or asthmatic serum containing various concentrations of omalizumab. Contractile responses to histamine or *Dermatophagoides pteronyssinus* (*D. pter*) were recorded using an organ bath system and expressed as percentage of maximal contractile response to acetylcholine (ACh). Immunohistochemistry was performed using monoclonal antibodies directed against IgE or tryptase. Mast cells were classified as fully granulated (type I), partly (type II) or largely degranulated (type III).

Results: The specific bronchial hyperresponsiveness to *D. pter* and the nonspecific bronchial hyperresponsiveness to histamine following passive sensitization were significantly inhibited by omalizumab in both distal and proximal airways. Passive sensitization-induced increase in IgE positive cells was also abolished by omalizumab in a concentration dependent manner. Mast cell degranulation which was inhibited by omalizumab was positively correlated with the contractile response to *D. pter*.

Conclusions: Omalizumab blocks specific and nonspecific bronchial hyperresponsiveness. Anti-IgE also decreases IgE bearing cell number and mast cell degranulation.

P. Berger¹, E. Scotto-Gomez¹,
M. Molimard², R. Marthan¹,
V. Le Gros³, J. M. Tunon-de-Lara^{1,4}

¹Laboratoire de Physiologie Cellulaire Respiratoire, Université Victor Segalen Bordeaux 2, INSERM E356, Bordeaux; ²Laboratoire de Pharmacologie, Université Victor Segalen Bordeaux 2, INSERM U657, Bordeaux; ³Novartis Pharma, Rueil Malmaison; ⁴CHU de Bordeaux, Hôpital Haut Lévêque, Service des Maladies Respiratoires, Pessac, France

Key words: asthma; bronchial hyperresponsiveness; immunoglobulin E; mast cell; smooth muscle.

J. Manuel Tunon-De-Lara
Laboratoire de Physiologie Cellulaire Respiratoire,
INSERM E356
Université V. Ségalen – Bordeaux 2
146 rue Léo Saignat
F-33076 Bordeaux Cedex
France

Accepted for publication 14 August 2006

In asthmatic patients, both symptoms and hyperresponsiveness are closely related to immunoglobulin E (IgE) concentration in serum (1, 2). Several immunohistological studies of bronchial biopsies performed in asthmatic patients have reported an increase in inflammatory cells bearing IgE receptor when compared with normal controls (3, 4) or nonatopic asthmatics (5). In this respect, the use of the anti-IgE monoclonal antibody omalizumab in asthmatic patients is particularly interesting. Omalizumab has been demonstrated to induce a reduction in inhaled corticosteroid dosage while improving peak flows and reducing exacerbations, particularly in patients at high-risk of serious asthma-related morbidity (6–12). When added to existing therapies of patients with more severe asthma, omalizumab also improved control of asthma (13). Djukanovic and colleagues have evaluated

the effect of omalizumab on airway inflammation in mild to moderate asthmatic patients (14). These authors observed in actively treated patients a significant reduction in eosinophil infiltration, but surprisingly, they did not find any effect of omalizumab on nonspecific airway hyperresponsiveness.

In vitro, passive sensitization of human isolated airways with serum from allergic asthmatic patients provides a good opportunity to study the role of IgE in bronchial reactivity. It has been previously shown that passive sensitization with asthmatic serum containing a high concentration of IgE alters the mechanical response of human isolated airways to electrical field stimulation, nonspecific agonists such as histamine, KCl, tachykinins or relaxant compounds (15–18). It is also well known that, during the passive sensitization, there is an increase

in IgE positive cells and that mast cell is the main cell phenotype bearing the IgE molecule (19). In addition, we have previously demonstrated that passive sensitization induces a mast cell degranulation related with *in vitro* hyperresponsiveness (20). Using the 17-9 anti-IgE monoclonal antibody (mAb), Rabe and coworkers have abolished the specific responsiveness to allergen but did not alter the nonspecific hyperresponsiveness induced by histamine (21). These results are in agreement with *in vivo* observations (14) but require further experiments performed with omalizumab (22).

The aim of the present study is to analyze the effect of the monoclonal antibody omalizumab directed against IgE on passive sensitization-induced (i) hyperresponsiveness, (ii) alterations in IgE positive inflammatory cells, and (iii) mast cell degranulation within the bronchial wall.

Methods

Patients and tissue preparation

Human lungs were obtained from 10 nonatopic and nonasthmatic patients undergoing thoracotomy as previously described (19, 20). All patients gave their written informed consent to participate to the study, after the nature of the procedure had been fully explained. The study received the approval from the local ethics committee. Patient characteristics are presented in Table 1. From a macroscopically tumor-free part of each of the specimens, segments of human bronchus (3rd to 7th generation) were carefully dissected and cut into rings measuring 4–5 mm in length. Proximal airways, i.e. 3–5 mm in internal diameter were separated from peripheral airways, i.e. 0.5–1.5 mm in internal diameter for functional study. For each lung specimen, one ring 1–2 mm in internal diameter was cut into small fragments for immunohistochemistry. Sera from healthy nonasthmatic and nonallergic subjects or from asthmatic and allergic patients [sensitized to *Dermatophagoides pteronyssinus* (*D. pter*)], whose characteristics are reported in Table 2, were initially incubated for 1 h at 37°C with 0, 60, 120 or 180 µg/ml of anti-IgE monoclonal antibody omalizumab (E25, Novartis Pharma, Rueil Malmaison, France). Passive sensitization was then processed as previously described (20) using an overnight incubation at room temperature with healthy nonasthmatic serum for control nonsensitized tissues (NS), or with asthmatic serum containing high levels

Table 2. Serum characteristics

Patient no.	Pathology	Sex (M/F)	Age (years)	FEV1 (%)	Eo (cell/mm) ³	total IgE (mUI)	RAST (SU/ml)	Skin test
Nonsensitized serum								
1	None	M	44	110	10	49	0	–
2	None	F	31	115	60	29	0	–
3	None	F	37	112	60	37	0	–
4	None	F	57	111	130	28	0	–
5	None	M	20	109	70	6	0	–
6	None	F	41	116	100	18	0	–
7	None	F	37	115	110	47	0	–
8	None	M	23	114	100	37	0	–
9	None	M	35	102	80	25	0	–
10	None	M	24	106	100	49	0	–
Asthmatic sera								
1	A + R	F	18	97	1070	100	3	+
2	A	M	40	110	nd	572	3	+
3	A + R	F	20	87	290	541	3	+
4	A	F	20	105	567	289	3	+
5	A + R	F	28	115	780	146	3	+
6	A + R	M	30	41	200	121	2	+
7	A	M	15	124	nd	515	3	+
8	A + R	F	23	140	nd	201	3	+

Pathology: A, asthma; R, rhinitis; FEV1, forced expiratory volume in 1 s; %, percentage of predicted values; Eo, eosinophil blood concentration; RAST (*D. pter*): class 0, < 1.4; class 1, 1.4–4; class 2, 4–20; class 3, 20–100; class 4, 100–300 SU/ml; nd, not determined.

of total and specific IgE to *D. pter* for passively sensitized (PS) tissues.

Mechanical recordings

Proximal and peripheral bronchial rings were mounted in an isolated organ bath system as previously described (20). Briefly, to determine the maximal contractile force, a supramaximal bolus of acetylcholine (ACh, Sigma, Saint-Quentin-Fallavier, France) (10^{-3} M) was administered to each ring. A cumulative concentration–response curve (CCRC) to histamine (10^{-8} to 10^{-3} M), (Sigma) was constructed for each ring. At the end of each experiment, 50 µl of allergen extract *D. pter* (Stallergenes, Antony, France, i.e. 0.1 µg/ml final concentration), to which serum IgE is directed, was added to the organ bath to evaluate the effect of passive sensitization.

Table 1. Patients characteristics

Patient no.	Histologic type	Sex (M/F)	Age (years)	Smoking (pack-yr)	FEV ₁ (%)	FEF _{25–75} (%)	sGaw (%)	TLC (%)	RV (%)	PO ₂ (mmHg)
1	SqCC	M	49	15	99	80	122	98	113	77
2	AdC	M	71	20	100	83	232	85	80	72
3	AdC	M	60	0	106	98	214	81	79	75
4	BP	M	55	40	69	52	69	136	208	73
5	SqCC	M	62	30	62	31	98	98	143	61
6	SqCC	M	73	50	82	87	34	74	55	69
7	AdC	M	56	60	110	80	117	113	139	85
8	AdC	M	81	0	69	42	51	100	145	78
9	SqCC	M	64	30	75	33	81	109	129	72
10	SqCC	M	73	0	87	53	39	93	101	68

SqCC, Squamous Cell Carcinoma; AdC, adenocarcinoma; BP, benign pathology; FEV₁, forced expiratory volume in 1 s; FEF_{25–75}, forced expiratory flow between 25% and 75% of forced vital capacity; sGaw, specific airway conductance; TLC, total lung capacity; RV, residual volume; %, percentage of predicted values; PO₂, arterial partial pressure of oxygen.

Sample processing and immunohistochemistry

Fragments of bronchial tissue prepared for immunohistochemistry were embedded in glycolmethacrylate (GMA) as described previously (19). Briefly, 2 μ m-thick GMA sections were incubated overnight with mouse mAb including anti-human IgE δ 1 (Immunotech, Marseille, France), anti-CD117 or anti-tryptase AA1 (Dako, Trappes, France). Detection system used a biotinylated secondary antibody (Dako) followed by the streptavidin-biotin horseradish-peroxidase complex (Dako) and the chromogen aminothyl carbamazole (Sigma).

Quantification of immunostaining

Light microscopy was performed using an Optiphot microscope (Nikon, Japan). Cells staining positively with each mAb were automatically counted by the software (Quancoul[®], Bordeaux, France) at a magnification of $\times 100$ in all of the specimen excluding alveoli and blood vessels as previously described (23). The total area examined was calculated by delineating the area of the bronchial section on a video interactive display system ($\times 40$). Cell counts were expressed as number of cells/mm² of tissue. We evaluate the mast cell degranulation using a validated method described previously (20, 23). Briefly, we evaluated the proportion of degranulated mast cells, by classifying the AA1 positive cells at a $\times 400$ magnification in three classes: I, fully granulated; II, partially degranulated; and III, largely degranulated.

Statistical analysis

Statistical comparison of paired mean CCRC ($F_{\max}$, and responses to each concentration of the agonist) was carried out first using analysis of variance (two ways ANOVA) and using paired Student's *t*-tests, with a Bonferroni correction factor. Since the cell counts were not normally distributed, results were analyzed by means of Wilcoxon paired rank test to compare the number of cells in each experimental condition. The χ^2 -test was used to compare the percentage of each experimental condition for each type of mast cell degranulation. Spearman correlation coefficients were determined to establish the relationship between mechanical responses and cell counts. Results were considered significant when $P < 0.05$.

Results

Functional results

Isolated airways passively sensitized with asthmatic serum containing a high concentration of specific IgE develop a contractile response to the corresponding antigen (i.e. *D. pter*), whereas bronchi incubated with serum from healthy volunteers do not (86.7 ± 20.8 vs. $2.5 \pm 2.5\%$ ACh, $P = 0.01$). In addition, incubation of isolated airways with asthmatic serum induces a hyper-responsiveness in terms of maximal contractile response to nonspecific agonists (i.e. histamine). For instance, CCRC obtained in distal airways are presented (Fig. 1A). The maximal contractile response to histamine was 132.5 ± 26.6 and $85.4 \pm 10.6\%$ ACh, for passively sensitized and nonsensitized airways ($P < 0.05$). This bronchial hyperresponsiveness to histamine was abolished by the previous incubation of the sensitizing

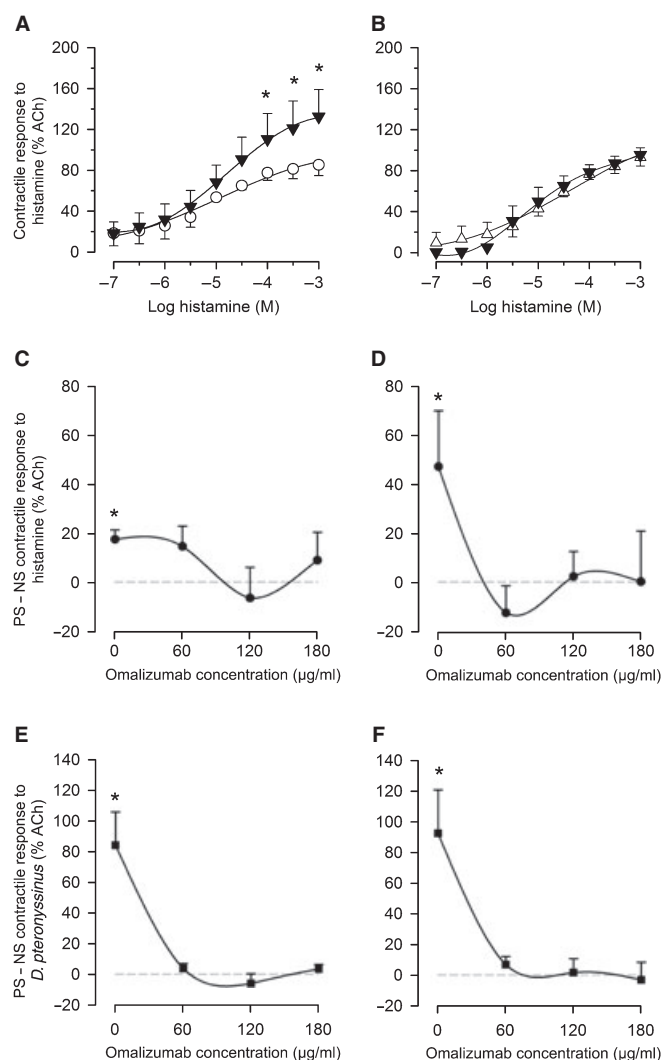


Figure 1. Mean cumulative concentration-response curves to histamine assessed by the percentage of maximal contractile response to ACh (% ACh) in distal bronchi preincubated with normal serum (nonsensitized, white symbols) or asthmatic serum (passively sensitized, black symbols) are presented in the absence ($n = 4$, A) or in the presence of 120 μ g/ml omalizumab ($n = 5$, B). Concentration-dependent effects of omalizumab assessed by the difference between passively sensitized and nonsensitized (PS-NS) bronchial contractile responses induced by histamine (C, D) or *D. pter* (E, F) using proximal ($n = 8$, C, E) or distal airways ($n = 5$, D, F). Values are mean $\pm$ SEM. * $P < 0.05$ using paired Student *t*-tests comparing passively and nonsensitized conditions.

serum for 60 min with omalizumab at the concentration of 120 μ g/ml (Fig. 1B). Furthermore, the concentration-dependent curves of omalizumab showed that these effects were maximal at the concentrations of 120 and 60 μ g/ml, for proximal and distal airways, respectively (Fig. 1C, D). A previous incubation of the sensitizing serum with omalizumab also abolished the specific

contractile response of proximal (Fig. 1E) and distal airways (Fig. 1F) to *D. pter*.

Immunohistochemistry

In terms of inflammation, passive sensitization of isolated bronchi with asthmatic serum with high concentration of IgE induced a significant increase in IgE positive cells [11.4 (1.2–31.8) vs. 17.1 (9.5–50.5) cell/mm² as median with 95% confidence interval in parenthesis, $P = 0.02$, paired wilcoxon]. A previous incubation with omalizumab abolished such increase in a concentration-dependent manner as assessed by the mean difference in IgE positive cell number between passively sensitized and nonsensitized tissues (Fig. 2A). Above 60 µg/ml of omalizumab, the number of IgE positive cells was not significantly different in sensitized and nonsensitized specimens.

In terms of mast cell degranulation, we found that passive sensitization induced a significant decrease in the percentage of fully granulated mast cells (Fig. 2B) with a concomitant increase in that of largely degranulated mast cells (Fig. 2C). Such mast cell subtypes are illustrated (Fig. 3). The previous incubation of sera with omalizumab abolished mast cell degranulation, since both the percentages of type I and type III mast cells were unchanged after passive sensitization (Fig. 2B and C).

Finally, we have evaluated the relationships between mast cell degranulation within the bronchial wall and the contractile response of the same bronchi induced by either histamine or *D. pter* (Table 3). The concentration of fully granulated type I mast cells in passively sensitized bronchi was negatively correlated with the histamine contractile shift induced by passive sensitization ($r = -0.50$, $P = 0.01$, Fig. 4A). The concentration of largely degranulated type III mast cells in passively sensitized bronchi was positively correlated with the *D. pter* shift induced by passive sensitization ($r = 0.49$, $P = 0.01$, Fig. 4B).

Discussion

Taken together these results demonstrate that omalizumab abolishes both specific and nonspecific hyperresponsiveness *in vitro*. This effect is related with the inhibition of mast cell degranulation observed within the airway wall of isolated human bronchi.

Passive sensitization of human airways is well known to induce a bronchial hyperresponsiveness *in vitro* (15–18). In this study, we confirm that passive sensitization increases the responsiveness to both *D. pter* and histamine. In addition, it has been clearly demonstrated that serum IgE level *in vivo* predicts bronchial reactivity *in vitro* (24). Therefore, we analyze the effect of omalizumab, a humanized monoclonal antibody neutralizing circulating free IgE on hyperresponsiveness induced by

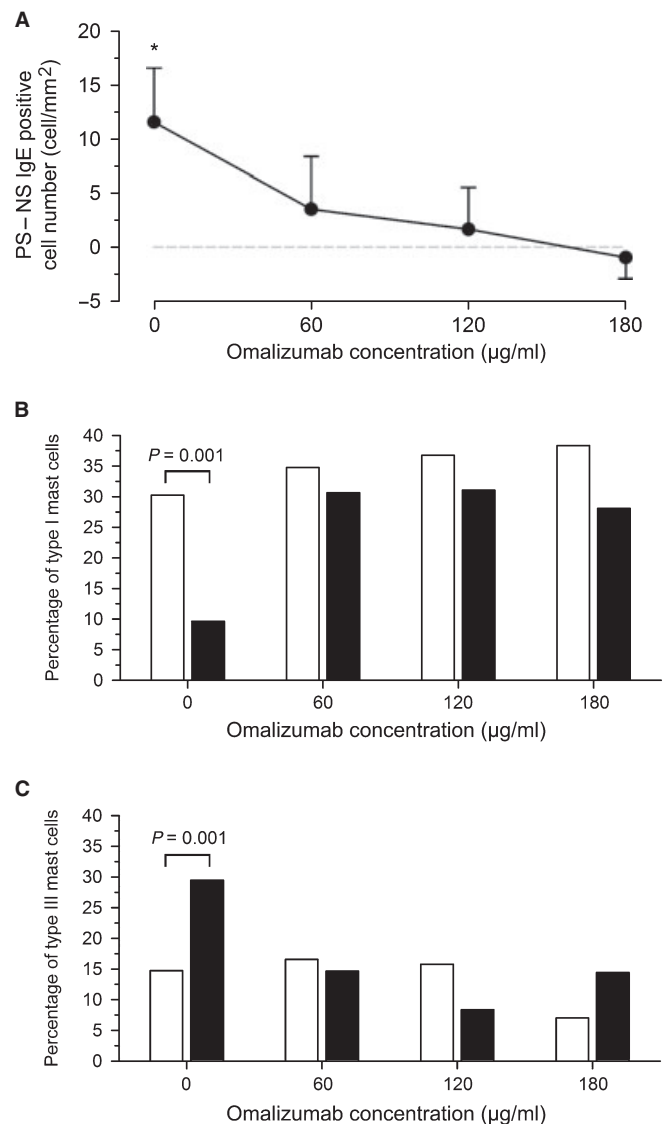


Figure 2. Concentration-dependent effects of omalizumab on the number of IgE bearing cells (A), the percentage of fully granulated (type I, B) and largely degranulated (type III, C) mast cells. (A) Values are the mean differences $\pm$ SEM ($n = 8$) between the number of IgE positive cells within the wall of passively sensitized and nonsensitized (PS-NS) bronchi. * $P < 0.05$ (Wilcoxon rank tests comparing passively and nonsensitized conditions). (B and C) Values are the percentages of mast cells in bronchi incubated with normal serum (nonsensitized, white bars, $n = 1824$ cells analyzed from nine specimens) and that of asthmatic serum (passively sensitized, black bars, $n = 1648$ cells analyzed from nine specimens). P -values are indicated when χ^2 tests were significant when comparing passively and nonsensitized conditions.

passive sensitization. Using 120 µg/ml of omalizumab reduced the responsiveness of proximal airways to histamine and similar results were obtained with lower concentrations of omalizumab (60 µg/ml) in distal airways. These results are in agreement with those reported

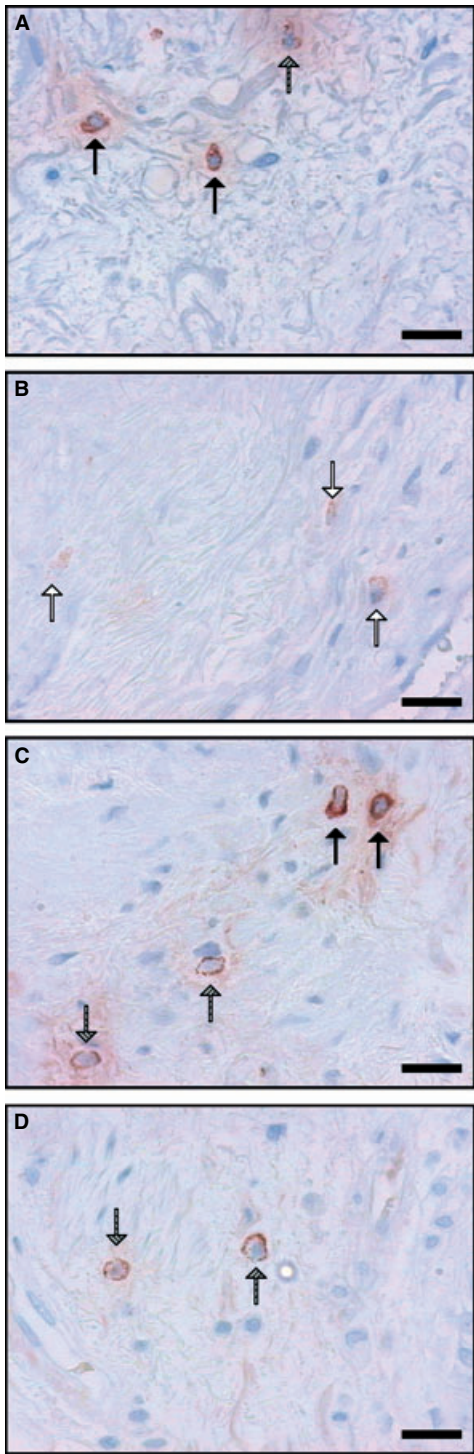


Figure 3. Optic microscopic views (magnification ×400) of isolated bronchi incubated with normal serum (nonsensitized, A and C) or asthmatic serum (passively sensitized, B and D). Such incubations are performed in the absence (A and B) or in the presence of 120 µg/ml of omalizumab (C and D). Sections are stained with anti-human tryptase antibody. Mast cells are classified as fully granulated (type I, black arrow), partially degranulated (type II, gray arrow), or largely degranulated (type III, white arrow). Bars represent 20 µm.

Table 3. Correlations between mechanical responses and immunohistochemical data

	Histamine (A–N) (% ACh)	P-value	<i>D. pter</i> (A–N) (% ACh)	P-value
Type I MC (N)	0.33	0.11	–0.15	0.46
Type I MC (A)	–0.50	0.01*	–0.51	0.01*
Type I MC (A–N)	–0.70	<0.001*	–0.29	0.16
Type II MC (N)	0.22	0.29	–0.04	0.84
Type II MC (A)	–0.25	0.25	–0.05	0.80
Type II MC (A–N)	–0.38	0.07	–0.13	0.53
Type III MC (N)	0.08	0.71	0.03	0.88
Type III MC (A)	0.20	0.34	0.49	0.01*
Type III MC (A–N)	0.16	0.45	0.28	0.17
IgE + cells (N)	0.11	0.60	–0.10	0.63
IgE + cells (A)	0.11	0.59	0.34	0.09
IgE + cells (A–N)	0.04	0.84	0.44	0.03*

MC, mast cells. Tissues were incubated with normal (N) or asthmatic (A) serum. Values are Spearman correlation coefficients with *P*-values when cell counts (cell/mm²) were plotted against mechanical responses induced by either histamine or *D. pter* (% ACh) in proximal airways.

by Fahy and co-workers demonstrating that nonspecific hyperresponsiveness induced by allergen challenge *in vivo* was inhibited by the previous administration of anti-IgE (25). In addition, it is well known that allergen challenge induces mast cell degranulation in bronchial mucosa that contributes to hyperresponsiveness during the late phase response (26). However, the ability of omalizumab to reduce hyperresponsiveness in patients suffering from mild to moderate asthma is more controversial. In a first study conducted by Bouley et al. methacholine PC20 improved slightly after 76 days of anti-IgE treatment (27) but more recent results obtained from a larger population of asthmatic patients failed to demonstrate a significant change of airway responsiveness to methacholine (14). However, hyperresponsiveness to indirect stimuli such as AMP is more closely related to underlying airway inflammation and symptoms of atopic asthma than responsiveness to direct stimuli such as methacholine (28). On the other hand, one could argue that the mechanism of baseline nonspecific bronchial hyperresponsiveness in asthma may be different from that depending on IgE following allergen challenge *in vivo* or passive sensitization *in vitro*. In a previous study, Rabe and colleagues reported that passive sensitization-induced specific hyperresponsiveness could be significantly inhibited by another anti-IgE monoclonal antibody (i.e. clone 17–9) (21). However, they failed to demonstrate any effect on nonspecific responsiveness to histamine. Omalizumab and 17–9 are two different clones of nonanaphylactogenic anti-human IgE with different properties that may affect IgE affinity.

Passive sensitization also induces an increase in IgE bearing cells (19, 20). In this study, we provide evidence that omalizumab reduces the number of IgE positive cells observed within the bronchial wall. We have previously shown that passive sensitization concomitantly induces a

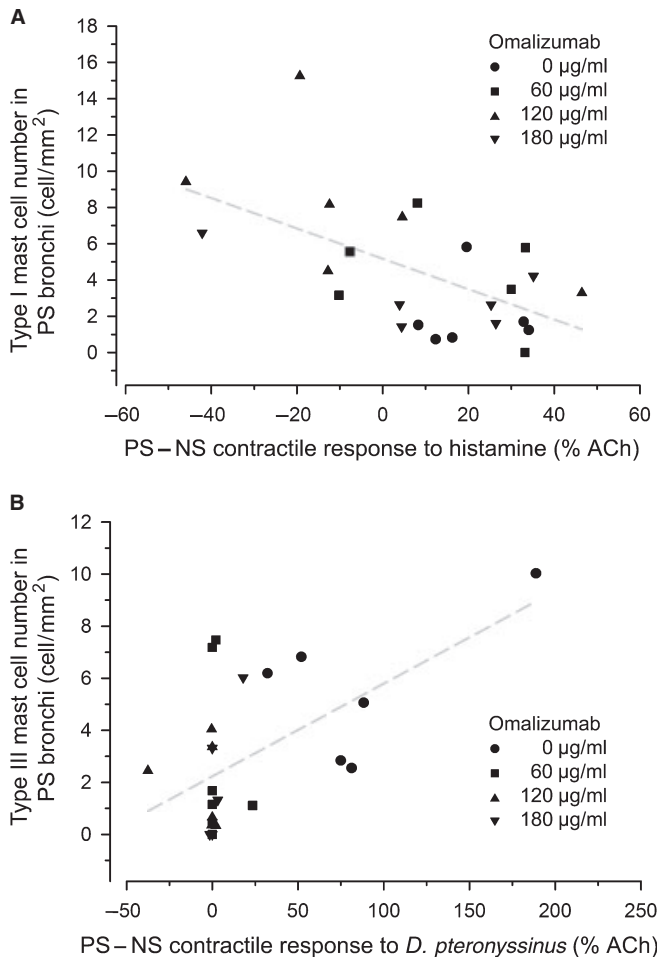


Figure 4. Correlations between the type I mast cell number in passively sensitized bronchi with the difference between passively sensitized and nonsensitized (PS–NS) bronchial contractile responses induced by histamine (A). Correlations between the type III mast cell number in passively sensitized bronchi with the difference between PS and NS bronchial contractile responses induced by *D. pter* (B). Sera are pretreated with various concentrations of omalizumab. Contractile responses are assessed by the percentage of maximal contractile response to ACh (% ACh).

mast cell degranulation in the absence of allergen (20). We speculated that monomeric IgE from asthmatic serum could be responsible for such mast cell degranulation. Previous experiments performed with mast cells have demonstrated that FcεRI could be activated by mono-

meric IgE leading to cytokine production and cell survival (29, 30). In addition, it has been recently shown that, in the absence of allergen, such monomeric IgE stimulation induces a dose-dependent histamine release, LTC₄ production and IL-8 synthesis by human mast cells (31). In the present study, using the anti-IgE omalizumab, mast cell degranulation is significantly decreased, confirming that IgE is the main factor involved in passive sensitization-induced mast cell degranulation.

Finally, we observed that mast cell degranulation was significantly correlated with *in vitro* hyperresponsiveness in terms of both maximal responses to histamine and to *D. pter*. These results are in agreement with those reported in atopic asthmatic children and adults. Bronchial hyperresponsiveness to histamine is correlated with mast cell degranulation in asthmatic patients assessed either by tryptase measurement in BAL fluid (32), or by immunohistochemistry of bronchial specimens, particularly during periods of exacerbation (33). Such relationship between hyperresponsiveness and mast cell activation has been confirmed by experiments performed *in vivo* with animal models of allergic asthma. Using FcεRIα chain deficient mice and different protocols of sensitization, Mayr and colleagues demonstrated that bronchial responsiveness was dependent on mast cell activation (34). In a model of IgE dependant-airway hyperresponsiveness induced by Respiratory Syncytial virus-specific IgE, another group observed a close relationship between methacholine response and mast cell degranulation (35).

Taken together, these results indicate that, by blocking IgE molecule, omalizumab abolishes mast cell degranulation and both specific and nonspecific human airway responsiveness. These observations confirm that passive sensitization induced mast cell degranulation with a release of tryptase (20), which can increase the bronchial responsiveness to nonspecific agonists (36). Further studies evaluating the effect of omalizumab on bronchial responsiveness are required *in vivo* and should include the analysis of mast cell degranulation.

Acknowledgments

The writers thank the staffs of Service de Chirurgie Thoracique, Hôpital Haut Lévêque, CHU de Bordeaux, for the supply of human lung tissue. This study was initiated and designed by the academic authors and supported by a grant from Novartis Pharma.

References

1. Burrows B, Martinez FD, Halonene M, Barbee RA, Cline MG. Association of asthma with serum IgE levels and skin-test reactivity to allergens. *N Engl J Med* 1989;**320**:271–277.
2. Sears MR, Burrows B, Flannery EM, Herbison GP, Hewitt CJ, Holdaway MD. Relation between airway responsiveness and serum IgE in children with asthma and in apparently normal children. *N Engl J Med* 1991;**325**:1067–1071.
3. Tunon de Lara JM, Redington AE, Bradding P, Church MK, Hartley JA, Semper AE et al. Dendritic cells in normal and asthmatic airways: expression of the α subunit of the high affinity immunoglobulin E receptor (Fc ϵ RI- α). *Clin Exp Allergy* 1996;**26**:648–655.
4. Poston RN, Chanez P, Lacoste JY, Litchfield T, Lee TH, Bousquet J. Immunohistochemical characterization of the cellular infiltration in asthmatic bronchi. *Am Rev Respir Dis* 1992;**145**:918–921.
5. Humbert M, Grant JA, Taborda-Barata L, Durham SR, Pfister R, Menz G et al. High affinity IgE receptor (Fc ϵ RI)-bearing cells in bronchial biopsies from atopic and nonatopic asthma. *Am J Respir Crit Care Med* 1996;**153**:1931–1937.
6. Soler M, Matz J, Townley R, Buhl R, O'Brien J, Fox H et al. The anti-IgE antibody omalizumab reduces exacerbations and steroid requirement in allergic asthmatics. *Eur Respir J* 2001;**18**:254–261.
7. Busse W, Corren J, Lanier BQ, McAlary M, Fowler-Taylor A, Cioppa GD et al. Omalizumab, anti-IgE recombinant humanized monoclonal antibody, for the treatment of severe allergic asthma. *J Allergy Clin Immunol* 2001;**108**:184–190.
8. Buhl R, Hanf G, Soler M, Bensch G, Wolfe J, Everhard F et al. The anti-IgE antibody omalizumab improves asthma-related quality of life in patients with allergic asthma. *Eur Respir J* 2002;**20**:1088–1094.
9. Buhl R, Soler M, Matz J, Townley R, O'Brien J, Noga O et al. Omalizumab provides long-term control in patients with moderate-to-severe allergic asthma. *Eur Respir J* 2002;**20**:73–78.
10. Holgate ST, Chuchalin AG, Hebert J, Lotvall J, Persson GB, Chung KF et al. Efficacy and safety of a recombinant anti-immunoglobulin E antibody (omalizumab) in severe allergic asthma. *Clin Exp Allergy* 2004;**34**:632–638.
11. Finn A, Gross G, van Bavel J, Lee T, Windom H, Everhard F et al. Omalizumab improves asthma-related quality of life in patients with severe allergic asthma. *J Allergy Clin Immunol* 2003;**111**:278–284.
12. Bousquet J, Cabrera P, Berkman N, Buhl R, Holgate S, Wenzel S et al. The effect of treatment with omalizumab, an anti-IgE antibody, on asthma exacerbations and emergency medical visits in patients with severe persistent asthma. *Allergy* 2005;**60**:302–308.
13. Humbert M, Beasley R, Ayres J, Slavin R, Hebert J, Bousquet J et al. 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;**60**:309–316.
14. Djukanovic R, Wilson SJ, Kraft M, Jarjour NN, Steel M, Chung KF et al. Effects of treatment with anti-immunoglobulin E antibody omalizumab on airway inflammation in allergic asthma. *Am J Respir Crit Care Med* 2004;**170**:583–593.
15. Marthan R, Crevel H, Guénard H, Savineau JP. Responsiveness to histamine in human sensitized airway smooth muscle. *Respir Physiol* 1992;**90**:239–250.
16. Black J, Marthan R, Armour CL, Johnson PR. Sensitization alters contractile responses and calcium influx in human airway smooth muscle. *J Allergy Clin Immunol* 1989;**84**:440–447.
17. Mitchell RW, Rühlmann E, Magnussen H, Leff AR, Rabe KF. Passive sensitization of human bronchi augments smooth muscle shortening velocity and capacity. *Am J Physiol* 1994;**267**:L218–L222.
18. Villanova X, Marthan R, Tunon de Lara JM, Johnson PRA, Savineau JP, Mc Kay KO, et al. Sensitization decreases relaxation in human isolated airways. *Am Rev Respir Dis* 1993;**148**:107–112.
19. Berger P, Walls AF, Marthan R, Tunon-de-Lara JM. Immunoglobulin E-induced passive sensitization of human airways: an immunohistochemical study. *Am J Respir Crit Care Med* 1998;**157**:610–616.
20. Berger P, N'Guyen C, Buckley M, Scotto-Gomez E, Marthan R, Tunon-De-Lara JM. Passive sensitization of human airways induces mast cell degranulation and release of tryptase. *Allergy* 2002;**57**:592–599.
21. Rabe KF, Watson N, Dent G, Morton BE, Wagner K, Magnussen H et al. Inhibition of human airway sensitization by a novel monoclonal anti-IgE antibody, 17–9. *Am J Respir Crit Care Med* 1998;**157**:1429–1435.
22. Tunon de Lara JM. Sensitization of human airways: what is the role of Immunoglobulin-E? *Clin Exp Allergy* 1998;**28**:660–663.
23. Berger P, Lavalée J, Rouiller R, Laurent F, Marthan R, Tunon-de-Lara JM. Assessment of bronchial inflammation using an automated cell recognition system based on colour analysis. *Eur Respir J* 1999;**14**:1394–1402.
24. Schmidt D, Watson N, Ruehlmann E, Magnussen H, Rabe KF. Serum immunoglobulin E levels predict human airway reactivity in vitro. *Clin Exp Allergy* 2000;**30**:233–241.
25. Fahy JV, Fleming HE, Wong HH, Liu JT, Su JQ, Reimann J et al. The effect of an anti-IgE monoclonal antibody on the early- and late-phase responses to allergen inhalation in asthmatic subjects. *Am J Respir Crit Care Med* 1997;**1155**:1828–1834.
26. Wenzel SE, Fowler AAD, Schwartz LB. Activation of pulmonary mast cells by bronchoalveolar allergen challenge. *In vivo* release of histamine and tryptase in atopic subjects with and without asthma. *Am Rev Respir Dis* 1988;**137**:1002–1008.
27. Boulet LP, Chapman KR, Cote J, Kalra S, Bhagat R, Swystun VA et al. Inhibitory effects of an anti-IgE antibody E25 on allergen-induced early asthmatic response. *Am J Respir Crit Care Med* 1997;**155**:1835–1840.
28. Currie GP, Saha S, Lee DK. Omalizumab and changes in airway hyperresponsiveness. *Am J Respir Crit Care Med* 2005;**171**:88–89.
29. Kalesnikoff J, Huber M, Lam V, Damen JE, Zhang J, Siraganian RP et al. Monomeric IgE stimulates signaling pathways in mast cells that lead to cytokine production and cell survival. *Immunity* 2001;**14**:801–811.
30. Asai K, Kitauro J, Kawakami Y, Yamagata N, Tsai M, Carbone DP et al. Regulation of mast cell survival by IgE. *Immunity* 2001;**14**:791–800.
31. Cruse G, Kaur D, Yang W, Duffy SM, Brightling CE, Bradding P. Activation of human lung mast cells by monomeric immunoglobulin E. *Eur Respir J* 2005;**25**:858–863.

32. Ferguson AC, Whitelaw M, Brown H. Correlation of bronchial eosinophil and mast cell activation with bronchial hyperresponsiveness in children with asthma. *J Allergy Clin Immunol* 1992;**90**:609–613.
33. Broide DH, Gleich GJ, Cuomo AJ, Coburn DA, Federman EC, Schwartz LB et al. Evidence of ongoing mast cell and eosinophil degranulation in symptomatic asthma airway. *J Allergy Clin Immunol* 1991;**88**:637–648.
34. Mayr SI, Zuberi RI, Zhang M, de Sousa-Hitzler J, Ngo K, Kuwabara Y et al. IgE-dependent mast cell activation potentiates airway responses in murine asthma models. *J Immunol* 2002;**169**:2061–2068.
35. Dakhama A, Park JW, Taube C, Chayama K, Balhorn A, Joetham A et al. The role of virus-specific immunoglobulin E in airway hyperresponsiveness. *Am J Respir Crit Care Med* 2004;**170**:952–959.
36. Berger P, Compton SJ, Molimard M, Walls AF, N'Guyen C, Marthan R et al. Mast cell tryptase as a mediator of hyperresponsiveness in human isolated bronchi. *Clin Exp Allergy* 1999;**29**:804–812.