
Effects of omalizumab and budesonide on markers of inflammation in human bronchial epithelial cells

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Background: Many patients with asthma have an IgE-mediated allergic component to the disease. Omalizumab, a monoclonal anti-IgE antibody, has demonstrated clinical efficacy in patients with allergic asthma. The effects of omalizumab on inflammation in asthma are not completely understood.

Objectives: To evaluate the effects of omalizumab on allergen- and growth factor-stimulated proinflammatory cytokine and nitric oxide (NO) production in human bronchial epithelial cells (BECs) and to compare them to the effects of budesonide, a corticosteroid with known anti-inflammatory properties.

Methods: Human BECs were stimulated in duplicate with interleukin 1 β (IL-1 β), 100 U/mL; ragweed, 10 μ g/mL; dust mite, 1,000 AU; and epithelial growth factor, 40 ng/mL; and either 10⁻⁷ M budesonide or 0.1 μ g/mL of omalizumab in a 4% dust mite atopic serum medium for 6 and 24 hours in 5% carbon dioxide at 37°C. Tumor necrosis factor α and transforming growth factor β expression and production and IL-4, IL-13, and NO production were assayed using gene-specific messenger RNA or sensitive enzyme-linked immunosorbent assays.

Results: Omalizumab inhibited the expression and of production proinflammatory cytokines and growth factor in antigen-stimulated BECs at 6 and 24 hours. Production of NO was inhibited at 6 hours and increased at 24 hours by omalizumab and budesonide.

Conclusions: The effects of omalizumab were similar to those of budesonide. These results, consistent with previously reported evidence of anti-inflammatory effects of omalizumab, demonstrate that omalizumab may reduce airway inflammation and probably contributes to decreased airway remodeling in patients with asthma.

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INTRODUCTION

IgE plays an important role in the early and late phases of allergic inflammation by binding to the high-affinity receptor (Fc ϵ RI) on the surface of mast cells and other immune cells.¹ The cross-linking of 2 surface-bound IgE molecules by allergen is followed by the release of cytokines responsible for the immediate and late-phase aspects of inflammation.²

Omalizumab (Xolair; Genentech Inc, South San Francisco, CA), a recombinant human monoclonal anti-IgE antibody, has demonstrated efficacy in patients with allergic asthma.^{3–5} Omalizumab attenuates early- and late-phase inflammatory responses by binding to and reducing free serum IgE and down-regulating Fc ϵ RI expression on human peripheral blood basophils.⁶ Research is ongoing to investigate the potential anti-inflammatory effects of omalizumab.

Several investigators recently examined the potential anti-inflammatory effects of omalizumab on airway inflammation in allergic asthma. Noga and colleagues⁷ attribute the anti-

inflammatory activity of omalizumab to decreased IgE levels, reduced cytokine (interleukin 13 [IL-13]) and cellular activation, and decreased peripheral eosinophil counts. Djukanovic and colleagues⁸ found that treatment with omalizumab decreases IgE levels in the airway; decreases markers of airway inflammation, including eosinophils and IL-4; and down-regulates Fc ϵ RI, the high-affinity receptor for IgE. Their findings demonstrate the anti-inflammatory activity of omalizumab.

In this study, we investigated the potential anti-inflammatory effects of omalizumab by measuring its effects on the production and expression of various cytokines, including tumor necrosis factor α (TNF- α), transforming growth factor β (TGF- β), IL-4, and IL-13, and on the production of nitric oxide (NO). Cytokine production and expression were measured in human bronchial epithelial cells (BECs) in a medium containing serum from a dust mite (DM)-sensitive individual and treated with various inflammatory mediators and antigens, including DM and ragweed. We compared the results with those for budesonide, a potent corticosteroid with known anti-inflammatory properties.

METHODS

Materials

Atopic serum from a DM-allergic individual was characterized using the enzyme-linked immunosorbent assay (ELISA)

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for specific IgE (Diagnostic Products Corp, Los Angeles, CA), and total IgE was evaluated using the VIDAS Total IgE Assay (bioMérieux Inc, Durham, NC) at Nassau University Medical Center Clinical Immunopathology Laboratory. Primary human BECs (NHBE 9527), bronchial epithelial growth medium (BEGM), trypsin/EDTA, HEPES-buffered saline solution, and trypsin neutralizing solution were purchased from Cambrex Bio Science (Walkersville, MD). Flavin adenine dinucleotide disodium, flavin mononucleotide, L-arginine, tetrahydrobiopterin, IL-1 β , epithelial growth factor (EGF), and bovine serum albumin were purchased from Sigma-Aldrich Corp (St Louis, MO). Ragweed and DM were obtained from Nelco Laboratories Inc (Deer Park, NY). Omalizumab was obtained from Genentech Inc, and budesonide was obtained from AstraZeneca Pharmaceuticals LP (Wilmington, DE). Tissue culture plates (24 wells) were purchased from Becton Dickinson (Franklin Lakes, NJ), and 25-cm² tissue culture flasks were purchased from Corning Inc (Corning, NY). The IL-4, IL-13, TNF- α , and TGF- β ELISAs and the Quantikine messenger RNA (mRNA) kits were purchased from R&D Systems (Minneapolis, MN). Colorimetric nonenzymatic NO assay was obtained from Oxford Biomedical Research (Oxford, MI).

Cell Culture

Human BECs were cultured at 37°C in BEGM. After 80% confluence, BECs were subcultured, rinsed with 5 mL of HEPES buffered saline solution per 25 cm², covered with 2 mL of trypsin/EDTA per 25 cm², neutralized by 4 mL of trypsin neutralizing solution per 25 cm², centrifuged at 220g for 5 minutes, and then resuspended in BEGM. The BECs were then seeded into 24-well plates at 1×10^5 cells/mL. After the BECs reached 80% confluence, the medium was changed and 4% atopic serum from a DM-allergic individual was added. The total IgE concentration was 8 IU/mL. The cells were supplemented with 10-mmol/L flavin adenine dinucleotide disodium, 5 μ M flavin mononucleotide, 0.1 μ M tetrahydrobiopterin, and 2-mmol/L L-arginine. They were also cultured with IL-1 β , 100 U/mL; ragweed, 10 μ g/mL, or DM, 1,000 AU/mL; and EGF, 40 ng/mL, in the absence or presence of 10⁻⁷M budesonide per well or 0.1 μ g of omalizumab per well. The BECs were incubated at 37°C in 4% DM atopic serum medium for 6 and 24 hours. Pellets and supernatants were then collected and stored at -70°C for the multiple cytokines' gene expression and production assay.

TNF- α and TGF- β mRNA Assay and ELISA

A sensitive Quantikine mRNA technique for human TNF- α and TGF- β was used to quantitate gene-specific mRNA at low levels. Samples of BEC lysate were hybridized with gene-specific biotin-labeled capture oligonucleotide probes and digoxigenin-labeled detection probes in a microplate. Results were recorded in attomoles per milliliter, where 1 amol (10⁻¹⁸ mol) $\approx$ 600,000 molecules. The cloned complementary DNA size for the TNF- α assay is 1,643 base pairs (bp). The cloned complementary DNA size for the TGF- β

assay is 2,745 bp. The sensitivity of the mRNA assay is 1.9 amol/mL for TNF- α and 2.0 amol/mL for TGF- β . Human TNF- α and TGF- β were analyzed in culture supernatants using a Quantikine ELISA immunoassay with microplates precoated with an anti-TNF- α monoclonal antibody and recombinant human anti-TGF- β soluble receptor II. The sensitivity of the assay is 1.6 pg/mL for TNF- α and 7.0 pg/mL for TGF- β .

IL-4 ELISA

Production of IL-4 was analyzed by Quantikine ELISA immunoassay using microplates precoated with a monoclonal antibody specific for IL-4. The assay uses an amplification system in which the alkaline phosphatase reaction provides a cofactor that activates a redox cycle, producing a color product. Briefly, alkaline phosphatase dephosphorylates nicotinamide adenine dinucleotide phosphate to reduced nicotinamide adenine dinucleotide (NADH), which serves as a specific cofactor that activates a redox cycle driven by alcohol dehydrogenase and diaphorase. The NADH reduces the tetrazolium salt to produce the colored formazan dye and nicotinamide adenine dinucleotide that is reduced by ethanol to regenerate NADH, which reenters the redox cycle. The sensitivity of the assay is 0.13 pg/mL.

NO Assay

Cadmium beads were washed twice in microcentrifuge tubes with 1 mL of water per tube, followed by 0.1M hydrochloric acid and 0.1M ammonium hydroxide before drying. Samples (50 μ L) were deproteinized by adding 140 μ L of water plus 10 μ L of 30% zinc sulfate. After mixing, samples were incubated at room temperature for 15 minutes and centrifuged at 4,000 rpm for 5 minutes at 6°C. Conversion of nitrate to nitrite was allowed to occur by transferring deproteinized samples to microcentrifuge tubes containing 6 to 7 cadmium beads followed by incubation at room temperature for 20 hours with agitation. The samples were then transferred to clean microcentrifuge tubes and were centrifuged at 4,000 rpm for 5 minutes at 6°C. Samples (50 μ L) and standards (0–100 μ M) were added to each well in 96-well plates, followed by the addition of 50 μ L of sulfanilamide. After shaking, 50 μ L of N-naphthyl-ethylenediamine dihydrochloride was added to each well and shaken for 5 minutes at room temperature. Absorbance was read on a microplate ELISA reader at a mean $\pm$ SD of 540 $\pm$ 20 nm. The sensitivity of this assay is 0.5 μ M.

Statistical Analysis

Data are expressed as mean $\pm$ SEM. Two-sample *t* tests and analysis of variance were calculated using a spreadsheet program (Excel version 2000; Microsoft Corp, Redmond, WA). A 2-sample *t* test assuming unequal variances and analysis of variance were applied to determine the differences among 22 conditions using a spreadsheet program (Excel). Differences associated with *P* $\leq$.05 were considered statistically significant.

RESULTS

Effects on TNF- α and TGF- β Expression and Production

The expression of TNF- α in BECs was undetectable at 6 and 24 hours. The production of TNF- α in BECs was also undetectable at 6 hours in control cultures and with all stimuli in the presence of omalizumab or budesonide. The addition of allergenic stimulants to control cultures (7.07 ± 0.8 pg/mL) increased TNF- α levels at 24 hours in cells stimulated with IL-1 β (18.1 ± 0.45 pg/mL; $P = .04$) and IL-1 β plus ragweed (13.22 ± 2.41 pg/mL; $P = .04$) (Fig 1). Omalizumab and budesonide significantly lowered TNF- α production in cells stimulated with IL-1 β ($P = 1.93 \times 10^{-5}$ and $P < .001$), ragweed ($P = .01$ and $P = .003$), and DM ($P = .01$ and $P = .02$) (Fig 1). However, TNF- α production was significantly enhanced by omalizumab in cells stimulated with EGF (3.69 ± 0.6 pg/mL vs 5.37 ± 0.4 pg/mL; $P = .03$) (Fig 1).

The addition of allergenic stimulants to control cultures increased TGF- β mRNA expression and production at 6 hours (Figs 2 and 3). Omalizumab inhibited TGF- β mRNA expression in all cultures at 6 hours, reaching statistical significance for IL-1 β (25.3 ± 1.08 pg/mL vs 22.76 ± 0.61 pg/mL; $P = .048$) (Fig 2). Omalizumab inhibited TGF- β production at 6 hours as well, reaching statistical significance for IL-1 β (929 ± 24.14 pg/mL vs 837 ± 11.9 pg/mL; $P = .01$), ragweed (910 ± 35.29 pg/mL vs 808 ± 36.54 pg/mL; $P = .046$), and EGF (925 ± 7.59 pg/mL vs 833 ± 14.4 pg/mL; $P = .001$) (Fig 3). Similarly, at 6 hours, budesonide significantly inhibited TGF- β mRNA expression stimulated by ragweed (23.48 ± 0.76 amol/mL vs 21.46 ± 0.46 amol/mL; $P = .04$), DM (23.38 ± 0.2 amol/mL vs 19.9 ± 0.58 amol/mL; $P = .002$), and IL-1 β plus DM (25.18 ± 0.17 amol/mL vs 21.99 ± 0.74 amol/mL; $P = .01$) (Fig 2) and significantly inhibited TGF- β production stimulated by rag-

weed (910 ± 35.29 pg/mL vs 835 ± 14.1 pg/mL; $P = .05$), IL-1 β plus DM (886.6 ± 14.6 pg/mL vs 847.3 ± 7.55 pg/mL; $P = .04$), and EGF (925 ± 7.59 pg/mL vs 865 ± 11.69 pg/mL; $P = .004$) (Fig 3).

At 24 hours, omalizumab significantly inhibited TGF- β mRNA expression in cells treated with IL-1 β (14.31 ± 0.55 amol/mL vs 12.47 ± 0.14 amol/mL; $P = .02$) but significantly increased expression in cells treated with EGF (18.12 ± 0.45 amol/mL vs 22.6 ± 0.47 amol/mL; $P = .0002$) (Fig 4). Omalizumab significantly inhibited TGF- β production in cells treated with IL-1 β ($1,525 \pm 25.83$ pg/mL vs $1,220 \pm 68.18$ pg/mL; $P = .007$), ragweed ($1,518 \pm 29.84$ pg/mL vs $1,403 \pm 43.54$ pg/mL; $P = .04$), and IL-1 β plus DM ($1,433 \pm 20.62$ pg/mL vs $1,328 \pm 24.84$ pg/mL; $P = .009$) for 24 hours (Fig 5). Budesonide significantly inhibited TGF- β mRNA expression in cultures treated with ragweed (12.88 ± 0.6 amol/mL vs 10.62 ± 0.73 amol/mL; $P = .03$), DM (13.16 ± 0.22 amol/mL vs 9.96 ± 0.46 amol/mL; $P = .002$), and IL-1 β plus ragweed (15.19 ± 0.76 amol/mL vs 12.46 ± 0.95 amol/mL; $P = .03$) for 24 hours (Fig 4) and inhibited TGF- β production, although the changes did not reach statistical significance (ragweed: $1,518 \pm 29.84$ pg/mL vs $1,418 \pm 56.5$ pg/mL; DM: $1,539 \pm 8.67$ pg/mL vs $1,457 \pm 49.51$ pg/mL; and IL-1 β plus ragweed: $1,470 \pm 27.72$ pg/mL vs $1,435 \pm 55.79$ pg/mL; $P =$ nonsignificant for all) (Fig 5).

Effects on IL-4 and IL-13 Production

The addition of allergenic stimulants to control cultures increased the production of IL-4 at 6 hours (Fig 6), but the changes were not statistically significant. Omalizumab inhibited IL-4 production stimulated by IL-1 β (0.12 ± 0.04 pg/mL vs 0.1 ± 0.02 pg/mL; $P =$ nonsignificant), ragweed ($0.17 \pm$

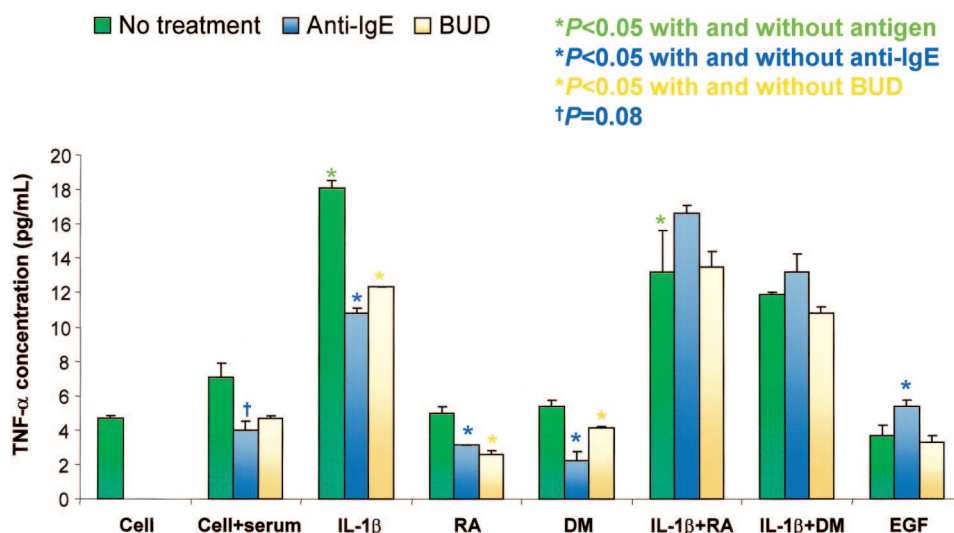


Figure 1. Mean tumor necrosis factor α (TNF- α) production from bronchial epithelial cells at 24 hours. Atopic serum at 4% for a total IgE concentration of 8 IU/mL was added to all conditions except the cell control. IL-1 β indicates interleukin 1 β ; RA, ragweed; DM, dust mite; EGF, epithelial growth factor; and BUD, budesonide. Error bars represent SEM. All samples were tested in quadruplicate. $P < .05$ was considered significant.

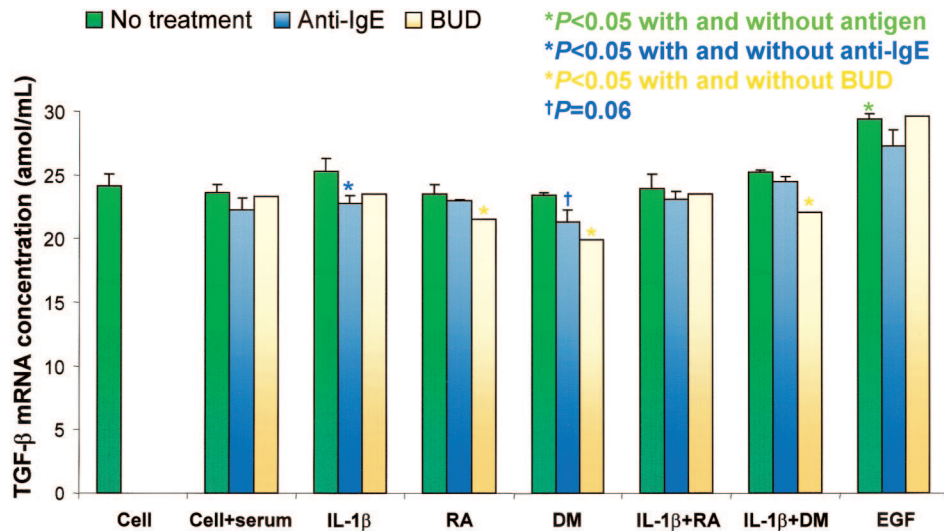


Figure 2. Mean transforming growth factor β (TGF- β) messenger RNA (mRNA) expression from bronchial epithelial cells at 6 hours. Atopic serum at 4% for a total IgE concentration of 8 IU/mL was added to all conditions except the cell control. IL-1 β indicates interleukin 1 β ; RA, ragweed; DM, dust mite; EGF, epithelial growth factor; and BUD, budesonide. Error bars represent SEM. All samples were tested in quadruplicate. $P < .05$ was considered significant.

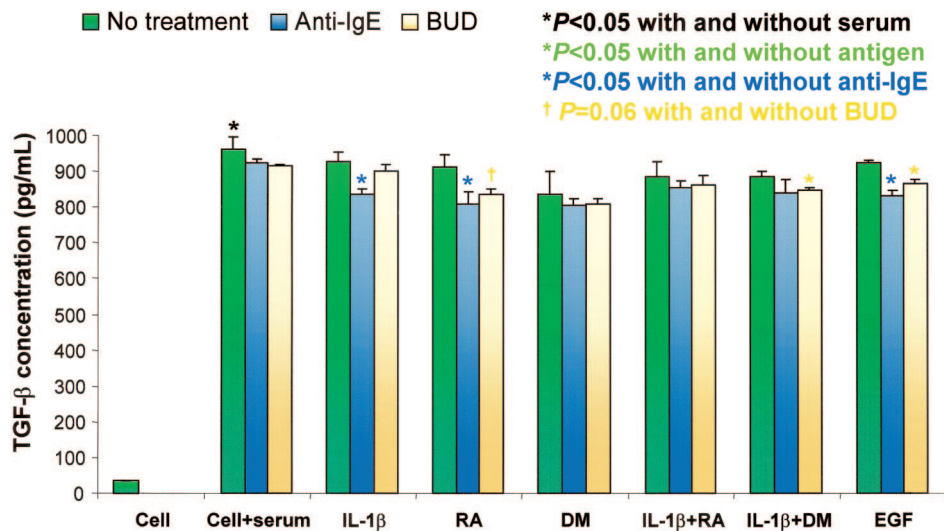


Figure 3. Mean transforming growth factor β (TGF- β) production from bronchial epithelial cells at 6 hours. Atopic serum at 4% for a total IgE concentration of 8 IU/mL was added to all conditions except the cell control. IL-1 β indicates interleukin 1 β ; RA, ragweed; DM, dust mite; EGF, epithelial growth factor; and BUD, budesonide. Error bars represent SEM. All samples were tested in quadruplicate. $P < .05$ was considered significant.

0.03 pg/mL vs 0.11 ± 0.02 pg/mL; $P =$ nonsignificant), IL-1 β plus ragweed (0.19 ± 0.04 pg/mL vs 0.15 ± 0.04 pg/mL; $P =$ nonsignificant), IL-1 β plus DM (0.21 ± 0.07 pg/mL vs 0.13 ± 0.04 pg/mL; $P =$ nonsignificant), and EGF (0.29 ± 0.11 pg/mL vs 0.07 ± 0.02 pg/mL; $P = .07$) at 6 hours. Omalizumab enhanced IL-4 production in DM-treated cultures (0.23 ± 0.06 pg/mL vs 0.32 ± 0.03 pg/mL), but the change was not significant. Similarly, budesonide inhibited IL-4 production in the presence of most stimulants. However, these changes also did not reach statistical significance. Interleukin 4 was not detected in any antigen-stimulated or

control cultures at 24 hours in the presence of either omalizumab or budesonide; IL-13 was also undetectable at 6 and 24 hours in all antigen-stimulated and control cultures in the presence of either omalizumab or budesonide.

Effects on NO Production

As expected, the addition of allergenic stimulants to control cultures increased the production of NO at 6 hours (Fig 7) and at 24 hours (data not shown). Omalizumab significantly inhibited NO production in cells stimulated with IL-1 β ($12.71 \pm 1.67 \mu\text{M}$ vs $6.38 \pm 0.01 \mu\text{M}$; $P = .02$), ragweed ($10.75 \pm$

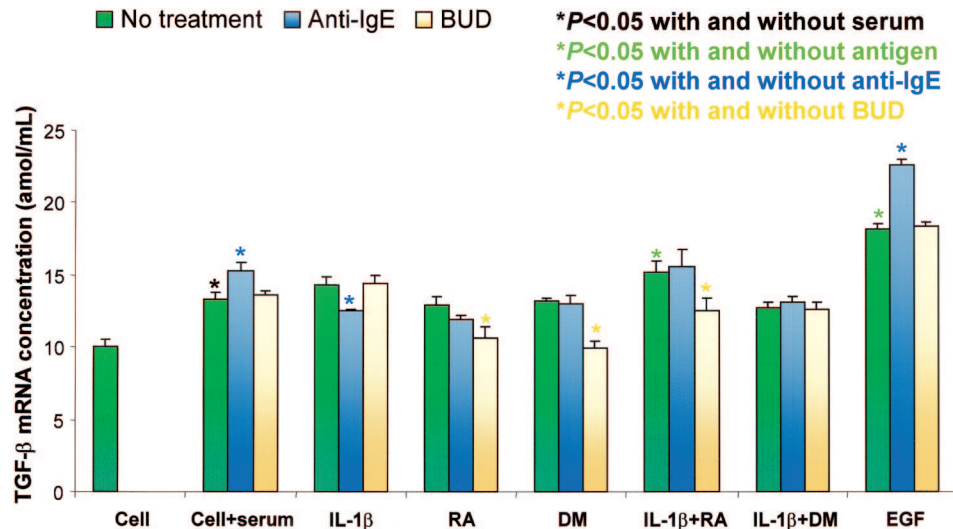


Figure 4. Mean transforming growth factor β (TGF- β) messenger RNA (mRNA) expression from bronchial epithelial cells at 24 hours. Atopic serum at 4% for a total IgE concentration of 8 IU/mL was added to all conditions except the cell control. IL-1 β indicates interleukin 1 β ; RA, ragweed; DM, dust mite; EGF, epithelial growth factor; and BUD, budesonide. Error bars represent SEM. All samples were tested in quadruplicate. $P < .05$ was considered significant.

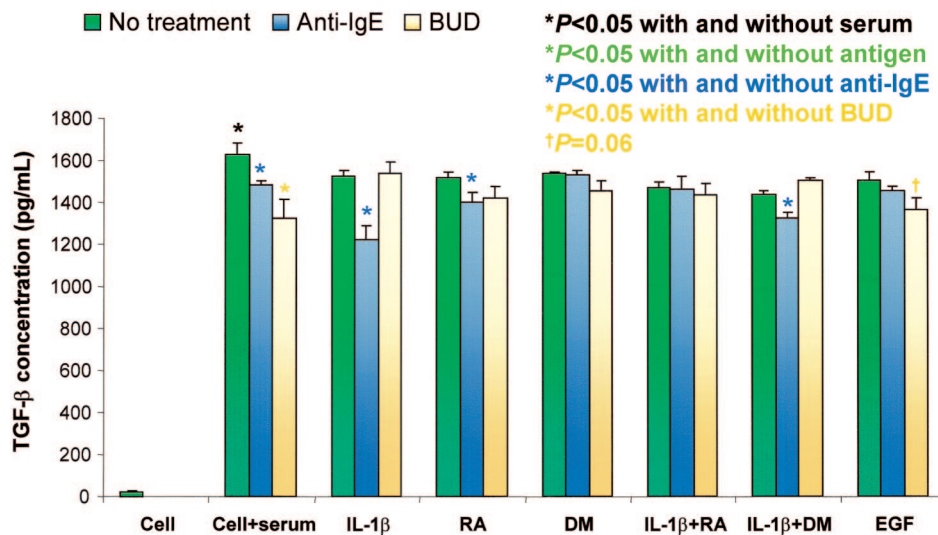


Figure 5. Mean transforming growth factor β (TGF- β) production from bronchial epithelial cells at 24 hours. Atopic serum at 4% for a total IgE concentration of 8 IU/mL was added to all conditions except the cell control. IL-1 β indicates interleukin 1 β ; RA, ragweed; DM, dust mite; EGF, epithelial growth factor; and BUD, budesonide. Error bars represent SEM. All samples were tested in quadruplicate. $P < .05$ was considered significant.

0.77 μ M vs 6.57 $\pm$ 1.23 μ M; $P = .02$), DM (8.88 $\pm$ 0.48 μ M vs 6.96 $\pm$ 0.66 μ M; $P = .03$), and EGF (8.11 $\pm$ 0.85 μ M vs 4.46 $\pm$ 0.73 μ M; $P = .009$) for 6 hours but significantly enhanced NO production in cells treated with IL-1 β plus ragweed (5.42 $\pm$ 0.37 μ M vs 16.55 $\pm$ 3.66 μ M; $P = .03$) for 6 hours (Fig 7) and with IL-1 β (1.4 $\pm$ 0.33 μ M vs 12.75 $\pm$ 3.08 μ M; $P = .02$), DM (4.67 $\pm$ 0.84 μ M vs 10.86 $\pm$ 1.19 μ M; $P = .004$), IL-1 β plus ragweed (3.69 $\pm$ 0.27 μ M vs 13.79 $\pm$ 3.49 μ M; $P = .03$), and IL-1 β plus DM (1.85 $\pm$ 0.01 μ M vs 11.55 $\pm$ 3.57 μ M; $P = .03$) for 24 hours. Similarly, budesonide significantly inhibited NO production stimulated by IL-1 β (12.71 $\pm$ 1.67 μ M vs 8.49 $\pm$

0.48 μ M; $P = .047$) and DM (8.88 $\pm$ 0.48 μ M vs 6.96 $\pm$ 0.37 μ M; $P = .01$) at 6 hours (Fig 7) and by EGF (7.52 $\pm$ 0.99 μ M vs 2.78 $\pm$ 0.52 μ M; $P = .004$) at 24 hours but significantly increased NO production stimulated by IL-1 β plus ragweed (5.42 $\pm$ 0.37 μ M vs 8.68 $\pm$ 0.31 μ M; $P = .0003$) at 6 hours (Fig 7) and IL-1 β plus DM (1.85 $\pm$ 0.01 μ M vs 5.88 $\pm$ 1.3 μ M; $P = .03$) at 24 hours.

DISCUSSION

In this study, omalizumab demonstrated a pattern of anti-inflammatory activity similar to that seen with budesonide.

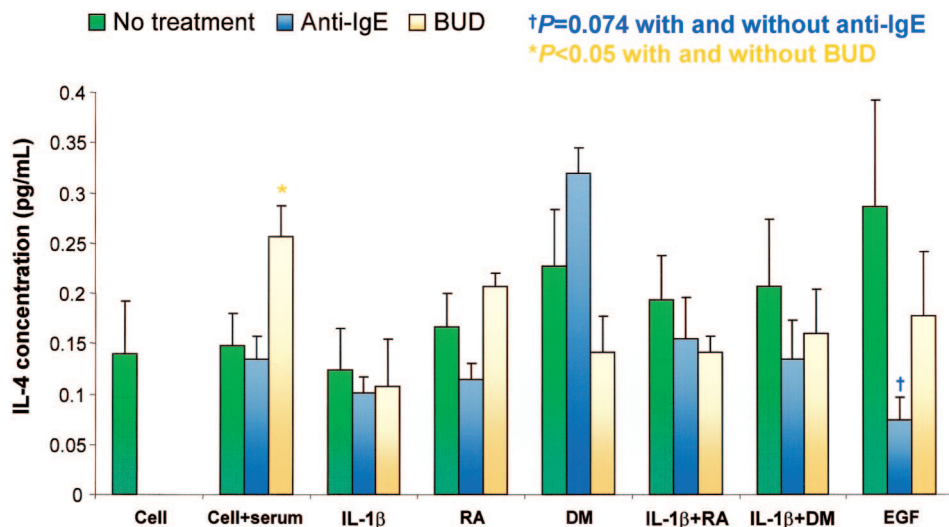


Figure 6. Interleukin 4 (IL-4) production from bronchial epithelial cells at 6 hours. Atopic serum at 4% for a total IgE concentration of 8 IU/mL was added to all conditions except the cell control. RA indicates ragweed; DM, dust mite; EGF, epithelial growth factor; and BUD, budesonide. Error bars represent SEM. All samples were tested in quadruplicate. $P < .05$ was considered significant.

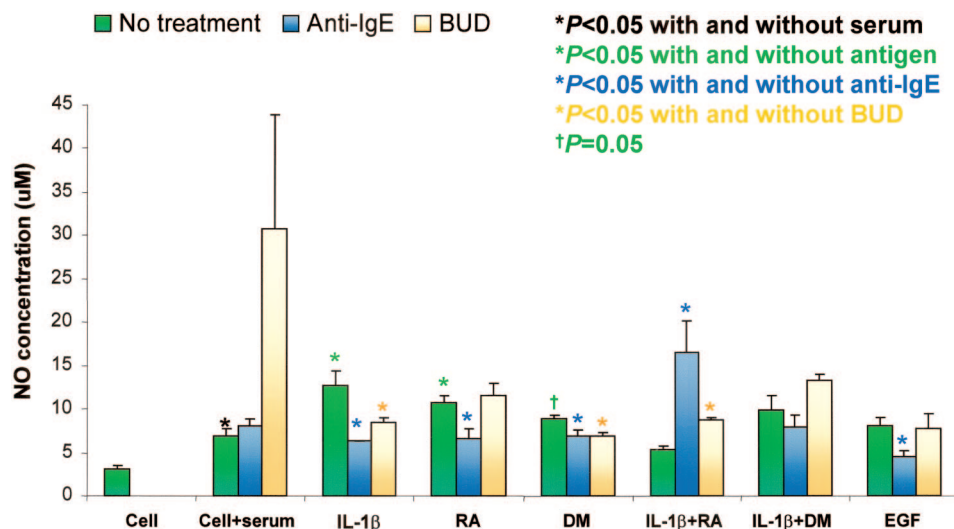


Figure 7. Mean nitric oxide production from bronchial epithelial cells at 6 hours. Atopic serum at 4% for a total IgE concentration of 8 IU/mL was added to all conditions except the cell control. IL-1β indicates interleukin 1β; RA, ragweed; DM, dust mite; EGF, epithelial growth factor; and BUD, budesonide. Error bars represent SEM. All samples were tested in quadruplicate. $P < .05$ was considered significant.

When added to BECs in a medium containing atopic serum and various allergenic stimulants, omalizumab decreased the production of $\text{TNF-}\alpha$, $\text{TGF-}\beta$, and IL-4 in most conditions. Human BECs were chosen because they play an important role in the allergic response and pathogenesis of asthma. In asthma, the bronchial epithelium is structurally and functionally abnormal. Structural changes, such as increased smooth muscle content and basement membrane thickening associated with matrix deposition, may contribute to persistent airway obstruction and bronchial hyperresponsiveness.⁹ Functional changes include increased expression and release

of proinflammatory cytokines¹⁰ and $\text{TGF-}\beta$.¹¹ Human BECs, which express the high-affinity IgE receptor ($\text{Fc}\epsilon\text{RI}$) on their surface, are activated by cross-linking of surface-bound IgE. A lower-affinity receptor for IgE, $\text{Fc}\epsilon\text{RII}$, is also expressed on the epithelial cells of some patients with asthma but not on nonasthmatic individuals.¹² However, Saini et al¹³ demonstrated that the $\text{Fc}\epsilon\text{RI}$ could be reversed on basophils in vitro by exposing the cells to IgE in short-term cultures. We hypothesize that a bronchial epithelial cell line could have the same effect. Therefore, we incubated the BECs with atopic serum, which contains free IgE in the cultures.

After human BECs were cultured in bronchial epithelial growth media, atopic serum from a DM-allergic individual was added. The cells were then cultured with various antigens and growth factors, including IL-1 β , ragweed, DM, and EGF, in the presence of either omalizumab or budesonide. The production of TNF- α , TGF- β , IL-4, IL-13, NO, and the expression of TNF- α and TGF- β mRNA were then measured as markers of inflammation after 6- and 24-hour incubation periods. The DM concentration of 1,000 AU/mL and the intervals of 6 and 24 hours were chosen based on the preliminary experiments at 6, 24, and 48 hours, which showed that BECs at 24 hours had the highest response for aggregation induced by DM antigen and a maximum response at 1,000 AU/mL.¹⁴ The concentration of omalizumab at 0.1 μ g/mL was chosen according to a study by MacGlashan et al,¹⁵ who used an anti-IgE dose response from 0.01 to 10 μ g/mL, which peaked close to 0.1 μ g/mL, for measuring histamine release in an in vitro-challenged basophil system.

Ragweed is a seasonal allergen and is used as a positive control for this study. Because the atopic serum was from a strongly DM-allergic individual tested by an in vitro specific IgE assay, significant activity in response to the addition of ragweed antigen was not expected. The atopic serum was not purified and also may have contained IgE antibodies sensitive to ragweed because the atopic donor was not skin tested and showed mild clinical symptoms during ragweed season.

Tumor necrosis factor α , an important cytokine in allergic airway reactions, is released by mast cells and eosinophils. It can promote inflammatory mediator and cytokine release, adhesion molecule expression, and granulocyte migration.¹⁶ Gene expression of TNF- α at 6 and 24 hours was undetectable. Production of TNF- α in BECs was also undetectable at 6 hours with all stimuli in the presence of omalizumab or budesonide. At 24 hours, omalizumab inhibited TNF- α production in most cells, reaching statistical significance for IL-1 β -, ragweed-, and DM-stimulated cells (Fig 1). Budesonide also significantly decreased TNF- α production in the same cells as omalizumab (Fig 1). The combination of IL-1 β and antigen or EGF alone showed increased TNF- α production with omalizumab. Because IL-1 functions together with TNF- α during innate immunity in promoting inflammation and innate immunity, the possibility exists that in the presence of antigens or growth factors, enhanced TNF- α production may occur. It is possible that omalizumab may have a beneficial effect in a mild state of inflammation, which could precede airway remodeling.

In allergic inflammation, the expression of TGF- β may be related to the fibrosis in asthmatic airways¹⁷ and airway remodeling in asthma.^{11,18,19} Omalizumab and budesonide had similar effects on TGF- β , inhibiting production and expression in most cells at 6 and 24 hours (Figs 2–5). At 6 hours, omalizumab inhibited TGF- β mRNA expression in all cells, reaching statistical significance for IL-1 β (Fig 2). Budesonide also inhibited TGF- β mRNA expression in all cells except EGF, reaching statistical significance for ragweed-, DM-, and IL-1 β plus DM-treated cells (Fig 2). Omalizumab

and budesonide also decreased TGF- β production in all stimulated cells at 6 hours (Fig 3). At 24 hours, omalizumab significantly inhibited TGF- β mRNA expression in cells treated with IL-1 β , whereas budesonide significantly inhibited TGF- β mRNA expression in cells treated with ragweed, DM, and IL-1 β plus ragweed (Fig 4). Omalizumab decreased TGF- β production at 24 hours in all stimulated cells, reaching statistical significance for IL-1 β , ragweed, and IL-1 β plus DM (Fig 5). Budesonide also decreased TGF- β production in most cells but not to the same extent as omalizumab (Fig 5).

Both IL-4 and IL-13 are important proinflammatory cytokines involved in the regulation of IgE¹⁷ that contribute to asthma severity and chronicity.^{19,20} Interleukin 4 exists in eosinophils and mast cells as a preformed, granule-associated peptide that can be released in allergic inflammatory responses.¹⁷ In the present study, omalizumab decreased IL-4 production in all stimulated cells at 6 hours except DM (Fig 6). Budesonide inhibited IL-4 production in all stimulated cells except ragweed. In both groups, IL-4 was not detected at 24 hours, and IL-13 was not detected at 6 or 24 hours. We realize that there are some inconsistent effects on various mediators and that perhaps additional intervals or longer incubation periods could have shown a more consistent effect.

Nitric oxide is a biomarker of airway hyperresponsiveness and inflammation.²¹ Because NO levels are significantly elevated in the exhaled air of patients with asthma,^{22,23} several investigators^{24–26} have evaluated exhaled NO as a noninvasive way to monitor inflammation in these patients. At 6 hours, omalizumab inhibited NO production in most stimulated cells, reaching statistical significance for IL-1 β , ragweed, DM, and EGF (Fig 7). Budesonide also decreased NO production in most cells but not to the degree seen with omalizumab (Fig 7). At 24 hours, however, NO production was increased in the presence of omalizumab and budesonide.

This difference in NO production at 6 and 24 hours with omalizumab and budesonide is somewhat confusing. Because NO is a well-established marker of inflammation, the suppression of NO at 6 hours is understandable and consistent with other results. The increase in NO production at 24 hours, however, is difficult to explain. Because NO levels reflect the presence of airway inflammation, known anti-inflammatory medications can alter NO production.^{27,28} The precise role of NO in the pathogenesis of asthma and allergic disease is still unknown.²⁹ Our apparently contradictory results may be attributable to the dichotomous role of NO, which can have proinflammatory and anti-inflammatory properties.³⁰ In the airways, NO may act as a bronchodilator and anti-inflammatory agent,²⁹ or it may have a detrimental role, amplifying inflammation by altering the T_H1/T_H2 balance toward the proallergic T_H2.³¹

Overall, the results seen with omalizumab are consistent with those seen with budesonide and provide evidence of an anti-inflammatory effect. The results are also consistent with those obtained by other investigators. Early studies confirmed that omalizumab suppresses the early- and late-phase re-

sponses to inhaled allergen in patients with allergic asthma. Fahy and colleagues³² found that omalizumab reduced serum IgE, increased the dose of allergen needed to provoke an early asthmatic response, and reduced the mean maximal fall in forced expiratory volume in 1 second during the early and late responses. Djukanovic and colleagues⁸ recently provided definitive evidence of the anti-inflammatory activity of omalizumab in a placebo-controlled study in 45 patients with corticosteroid-naïve, mild-persistent asthma using bronchial biopsy and induced sputum sampling. Patients were treated with omalizumab or placebo for 16 weeks and were evaluated with bronchial biopsy and induced sputum sampling. Treatment with omalizumab led to a significant decrease in serum IgE levels and a reduction in IgE-positive cells in the airway mucosa. The mean percentage sputum eosinophil count decreased significantly from 6.6% to 1.7% in the omalizumab group ($P < .001$), a reduction significantly greater ($P = .05$) than that in the placebo group (from 8.5% to 7.0%). The depletion of IgE from airway tissue was associated with a marked reduction in airway eosinophilia, expression of the FcεRI receptor, and the central T_H2 cytokine IL-4. Noga and colleagues⁷ also found that IL-13 levels and peripheral eosinophil counts decreased significantly in patients treated with omalizumab vs placebo.

The results of this study are consistent with previously published evidence of the anti-inflammatory effects of omalizumab and support the observed clinical efficacy in large-scale clinical trials. They provide further insight into the mechanisms through which anti-IgE therapy with omalizumab reduces airway inflammation and improves symptoms in patients with allergic asthma. In terms of a possible postulated mechanism of action, IgE antibodies are immune mediators of airway inflammation in allergic atopic asthma, and IgE-mediated hypersensitivity reactions are the likely mechanisms that may cause chronic airway inflammation in asthma through effector cells that release cytokines activated via high-affinity (FcεRI) IgE receptors on BECs.³³ Omalizumab may affect airway epithelium in proximity to mast cells and basophils and other effector cells, thereby altering cytokine production and expression from the airway epithelium in a downstream manner. It is also known that omalizumab treatment down-regulates dendritic cell FcεRI expression.³⁴ The clustering of IgE antibodies bound to FcεRI expressed by BECs may lead a signal transduction mechanism to up-regulate or down-regulate cytokine production or de novo gene expression.

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