

Increased IgE⁺ B Cells in Sputum, but Not Blood, Bone Marrow, or Tonsils, after Inhaled Allergen Challenge in Subjects with Asthma

To the Editor:

The allergic cascade is critically dependent on allergen-specific IgE. Although exposure to allergen is shown to directly induce IgE class switching in the airway of patients with asthma (1, 2), it is unknown whether elevated IgE levels (2, 3) are due to heightened activity of IgE⁺ B cells or expansion of the IgE⁺ B-cell population. We evaluated the kinetics of IgE⁺ B-cell subsets in the airway, circulation, and hematopoietic and lymphatic compartments after inhaled allergen challenge (clinicaltrials.gov identifier, NCT01420003). We recruited 19 nonsmoking subjects with stable allergic asthma (no exacerbations or asthma medications), with an FEV₁ of at least 70% and methacholine PC₂₀ not exceeding 16 mg/ml (4). Baseline methacholine PC₂₀ was measured, and we collected sputum from one subset (n = 7) and blood and bone marrow from another (n = 12). Subjects inhaled allergen until the FEV₁ dropped at least 20% from prechallenge, and FEV₁ was measured for 7 hours. Blood and sputum samples were again collected 7 hours, 24 hours, 7 days, 14 days, and 28 days postchallenge. Bone marrow was collected 7 and 28 days postchallenge. These methods have been previously described (5, 6). A tonsil biopsy sample (from n = 10 subjects who had tonsils) was collected 28 days postchallenge, mechanically disrupted, incubated in 1% collagenase for 2 hours, and filtered through a 100-μm filter. Subjects underwent inhaled diluent (control) challenge with all measurements repeated.

Sputum cells were surface stained with antibodies to CD45, CD4, CD8, and CD19 to enumerate B and T cells. Blood and tonsil cells were surface stained with CD45, CD19, CD38, CD27, and CD138 to enumerate memory B cells (CD45⁺CD19⁺CD27⁺CD38⁻) and plasmablasts (CD45⁺CD19⁺CD27⁺CD38⁺). Bone marrow cells were surface stained with antibodies to CD45, CD3, CD19, CD38, and CD138 to enumerate plasma cells (CD3⁻CD45⁺CD38⁺CD138⁺) and plasmablasts (CD3⁻CD45⁺CD38⁺CD138⁻). All cells were stained intracellularly for IgE (immunoglobulin of interest) and IgG (control). Cells were acquired with a BD LSRII flow cytometer (BD Biosciences, San Jose, CA) and analyzed with FlowJo software (TreeStar, Ashland, OR). CD19⁺ B cells and CD4⁺ and CD8⁺ T cells were expressed as a percentage of total lymphocytes, whereas B-cell subsets were expressed as a percentage of CD19⁺ B cells. Total IgE and B-cell activating factor were measured in sputum, and allergen-specific IgE was measured in serum and sputum.

Allergen inhalation induced early and late asthmatic responses (31 and 14% fall in FEV₁, respectively) and methacholine PC₂₀ decreased from 4.4 (95% confidence interval, 2.2–8.8) mg/ml before to 3.4 (95% confidence interval, 2.2–5.1) mg/ml 24 hours after allergen challenge. Sputum eosinophils increased from 6.1 ± 1.4% at baseline to 15.6 ± 3.3% (*P* < 0.05) 7 hours after allergen challenge, and CD4⁺ T cells also increased (Figure 1A), confirming the development of an allergic response in the airways.

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The frequency of sputum CD19⁺ B cells increased 7 hours after allergen challenge (*P* < 0.05), but the frequency of CD8⁺ T cells was unchanged (Figure 1A). The IgE⁺ B-cell frequency and number per gram of sputum had increased 7 hours after allergen challenge (*P* < 0.05), with no change in IgG⁺ B cells in this small sample size (Figure 1B). In sputum, the levels of B-cell activating factor increased 7 and 24 hours after allergen challenge (*P* < 0.05; Figure 2A). Total IgE increased 7 hours after allergen challenge (*P* < 0.05); however, the 138 ± 22% increase in allergen challenge-specific IgE in sputum 7 hours after allergen challenge was not different from diluent control (Figure 2A).

Baseline frequencies of IgE⁺ plasma cells and plasmablasts from bone marrow, and IgE⁺ memory B cells from blood and tonsils, were 0.8 ± 0.1, 1.0 ± 0.1, 0.3 ± 0.1, and 0.7 ± 0.3% as a percentage of CD19⁺ B cells, respectively. There were no changes in these cell frequencies after allergen challenge compared with diluent (data not shown). Furthermore, in peripheral blood and tonsil tissue the frequency of plasmablasts was too low to quantitate accurately.

Sputum CD19⁺ B cells negatively correlated with methacholine PC₂₀ (*r* = -0.65, *P* = 0.01) and positively correlated with sputum eosinophils (*r* = 0.53, *P* = 0.049) and CD8⁺ T cells (*r* = 0.61, *P* = 0.02). Sputum IgE⁺ B cells positively correlated with sputum CD4⁺ T cells (*r* = 0.63, *P* = 0.02).

Short-lived IgE⁺ B cells have been shown to transiently increase in the lungs, spleen, and bone marrow of mice 3 days after ovalbumin inhalation challenge, and long-lived IgE⁺ B cells increase in the bone marrow 30 days after challenge (7). We found an increased frequency of IgE⁺ B cells in the sputum after allergen inhalation but did not observe changes in tonsils, bone marrow, or peripheral blood. These different findings from mice could be due to the length of exposure to antigen, whereby ongoing exposure in subjects with allergic asthma may induce higher underlying B-cell responses, making it more difficult to see changes postchallenge. Furthermore, studies from various murine models suggest there are inherent differences in anatomic and dynamic molecular aspects of IgE class switching that render comparisons between mouse and human complex (8).

The coincident acute increases in IgE⁺ B cells and total IgE in sputum after allergen inhalation challenge suggest that local signals in the airway microenvironment may lead to a transient local expansion or influx of IgE⁺ B-cell subsets. Inhaled allergen had no acute effect in the circulation or the lymphatic and hematopoietic compartments, likely because class-switch recombination typically requires approximately 7–14 days to occur (9). The proliferation and expansion of CD19⁺ B cells and IgE⁺ B cells in the sputum 7 hours after allergen challenge also corresponded with increased sputum B-cell activating factor levels, a mediator implicated in B-cell proliferation and differentiation (10, 11) and produced by dendritic cells and airway epithelium (12).

A large proportion of IgE⁺ B cells, but not IgG⁺ B cells, are able to differentiate into plasma cells (13). Our data demonstrate an acute expansion of IgE⁺ B cells in the sputum, corresponding to higher levels of total IgE and B-cell activating factor. That allergen inhalation did not increase allergen-specific IgE in the sputum suggests that the increase in total IgE may be a result of polyclonal switching 7 hours after allergen challenge. The acute increase in local levels of total IgE and IgE⁺ B cells, taken together with the

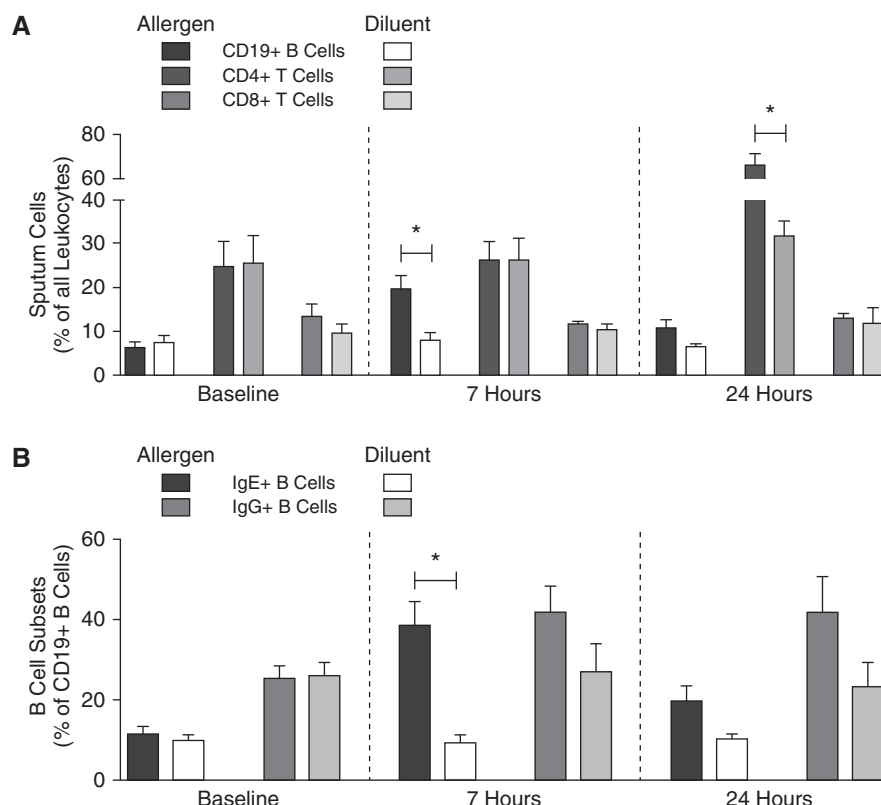


Figure 1. The effects of inhaled allergen on (A) sputum lymphocyte subsets expressed as a percentage of sputum leukocytes and (B) sputum B-cell subsets expressed as a percentage of sputum B cells, measured at baseline and again at 7 and 24 hours after allergen and diluent challenges ($n = 7$ subjects). Data are presented as means $\pm$ SEM. Significant findings are denoted by an asterisk, where $P < 0.05$ compared with diluent.

delayed increase in allergen-specific IgE in the serum 14 days postchallenge, may be explained by a prolonged time course of class-switch recombination and differentiation to allergen-specific plasma cells (9). Together, these results support a mechanism whereby eosinophilic airway inflammation and airway hyperresponsiveness are elevated after allergen challenge through local expansion of IgE⁺ B cells. ■

Author disclosures are available with the text of this letter at www.atsjournals.org.

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References

- Wong KJ, Timbrell V, Xi Y, Upham JW, Collins AM, Davies JM. IgE⁺ B cells are scarce, but allergen-specific B cells with a memory phenotype circulate in patients with allergic rhinitis. *Allergy* 2015;70:420–428.
- Wilson DR, Merrett TG, Varga EM, Smurthwaite L, Gould HJ, Kemp M, Hooper J, Till SJ, Durham SR. Increases in allergen-specific IgE in BAL after segmental allergen challenge in atopic asthmatics. *Am J Respir Crit Care Med* 2002;165:22–26.
- van de Pol MA, Lutter R, van Ree R, van der Zee JS. Increase in allergen-specific IgE and *ex vivo* Th2 responses after a single bronchial challenge with house dust mite in allergic asthmatics. *Allergy* 2012;67:67–73.
- Bousquet J, Clark TJH, Hurd S, Khaltayev N, Lenfant C, O'byrne P, Sheffer A. GINA guidelines on asthma and beyond. *Allergy* 2007;62:102–112.
- Gauvreau GM, Watson RM, O'Byrne PM. Kinetics of allergen-induced airway eosinophilic cytokine production and airway inflammation. *Am J Respir Crit Care Med* 1999;160:640–647.
- El-Gammal A, Oliveria J-P, Howie K, Watson R, Mitchell P, Chen R, Baatjes A, Smith S, Al-Sajee D, Hawke TJ, *et al*. Allergen-induced changes in bone marrow and airway dendritic cells in subjects with asthma. *Am J Respir Crit Care Med* 2016;194:169–177.
- Luger EO, Fokuhl V, Wegmann M, Abram M, Tillack K, Achatz G, Manz RA, Worm M, Radbruch A, Renz H. Induction of long-lived allergen-specific plasma cells by mucosal allergen challenge. *J Allergy Clin Immunol* 2009;124:819–826.e4.
- Wu LC, Scheerens H. Targeting IgE production in mice and humans. *Curr Opin Immunol* 2014;31:8–15.
- Ramadani F, Upton N, Hobson P, Chan Y-C, Mzinza D, Bowen H, Kerridge C, Sutton BJ, Fear DJ, Gould HJ. Intrinsic properties of germinal center-derived B cells promote their enhanced class switching to IgE. *Allergy* 2015;70:1269–1277.

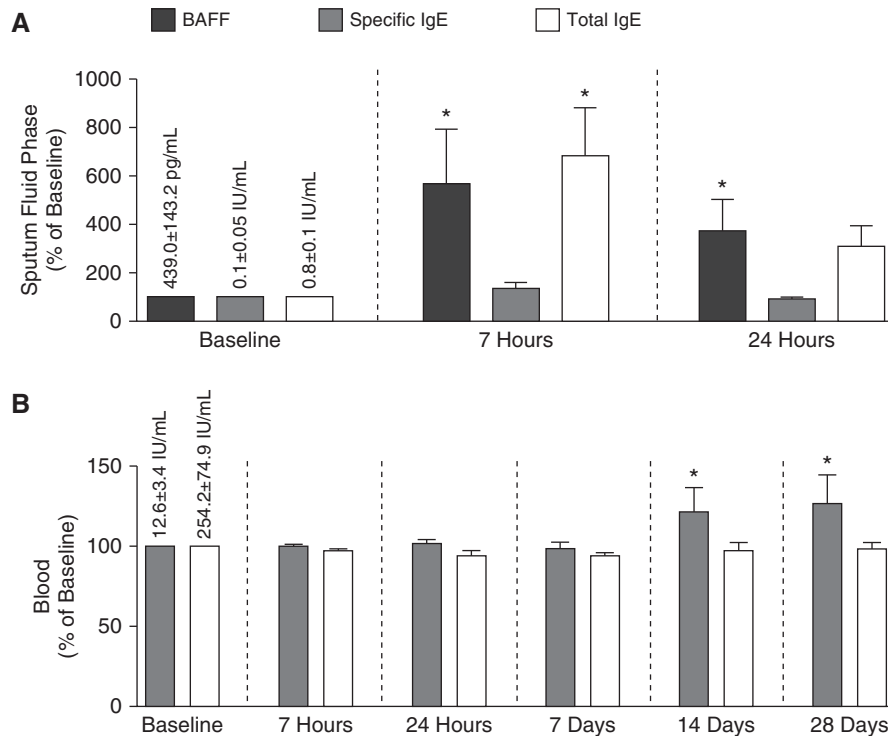


Figure 2. The effect of inhaled allergen on (A) B-cell activating factor (BAFF), specific IgE, and total IgE levels in sputum fluid phase measured at baseline and again at 7 and 24 hours after challenge ($n = 7$ subjects) and (B) specific IgE and total IgE levels in blood measured at baseline and until 28 days after challenge ($n = 12$ subjects). Data are presented as means $\pm$ SEM. Significant findings are denoted by an asterisk, where $P < 0.05$ compared with prechallenge baseline.

10. Mackay F, Browning JL. BAFF: a fundamental survival factor for B cells. *Nat Rev Immunol* 2002;2:465–475.
11. Samitas K, Malmhäll C, Rådinger M, Ramos-Ramírez P, Lu Y, Deák T, Semitekolou M, Gaga M, Sjöstrand M, Lötvall J, *et al.* Precursor B cells increase in the lung during airway allergic inflammation: a role for B cell-activating factor. *PLoS One* 2016;11:e0161161.
12. Kato A, Truong-Tran AQ, Scott AL, Matsumoto K, Schleimer RP. Airway epithelial cells produce B cell-activating factor of TNF family by an IFN- β -dependent mechanism. *J Immunol* 2006;177:7164–7172.
13. Ramadani F, Bowen H, Upton N, Hobson PS, Chan Y-C, Chen J-B, *et al.* Ontogeny of human IgE-expressing B cells and plasma cells. *Allergy* 2017;72:66–76.

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Putative Mechanisms of Action of Endobronchial Coils

To the Editor:

Chronic obstructive pulmonary disease is a progressive disease characterized by flow limitation, perceived most commonly as dyspnea (1). Although bronchodilators, still the cornerstone of

end-stage treatment, quite often are found inadequate to alleviate patients' symptoms, new procedures have been developed, including surgical techniques such as lung volume reduction surgery targeted to reduce resting hyperinflation and its static and dynamic components (2). The procedure improved elastic recoil, enhanced the radial traction of the airways, and finally increased conductance and expiratory flows (3). Bronchoscopic lung volume reduction methods are emerging as a viable alternative to lung volume reduction surgery (4–9). Endobronchial valves are most effective when they result in significant lung volume reduction by inducing atelectasis (4). Previous studies highlighted the safety and efficacy profile of the lung volume reduction coils (LVRCs) (5–9). In contrast, the mechanism of action of the procedure remains unclear. Recently, the RESET (Endobronchial Coils for the Treatment of Emphysema with Hyperinflation; ClinicalTrials.gov NCT01334307) trial concluded that there were no changes in total lung capacity (TLC) (5) and supported that the radial traction exerted by the LVRC created volume reduction in the targeted lung areas and tethered open the small airways (5, 9). To examine further the effects of LVRC, we have performed a more detailed analysis of the pulmonary function data obtained from a large subset of the patients in the RESET study and correlated them with changes in lobar lung volumes and emphysema scores obtained from computerized tomography (CT) scans.

Methods

The design, methods, and primary data of the RESET study have been published previously (5). This further analysis is based on the subgroup of patients enrolled only at the Royal Brompton and Harefield National Health Service Hospital Trust in a one-to-one

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