

Detecting Anti-FcεRI Autoantibodies in Patients with Asthma by Flow Cytometry

RS SUN^{1*}, XH CHEN², JF SUI^{3*}, RQ LIU¹, TM CHENG¹, XZ RAN¹ AND T YANG¹

¹Department of Dermatology, Institute of Battle Surgery, Daping Hospital, Third Military Medical University, Chongqing, China; ²The National Key Laboratory of Respiratory Disease, Guangzhou Institute of Respiratory Disease, First Affiliated Hospital of Guangzhou Medical College, Guangzhou, China; ³Department of Physiology, Third Military Medical University, Chongqing, China

Autoimmune diseases have been implicated in the development of intrinsic asthma, however little data are available on the role of autoimmunity in the pathogenesis of asthma. The purpose of this study was to investigate circulating autoantibodies against the high-affinity receptor for immunoglobulin E, FcεRI, in patients with asthma. Seventy-eight patients with asthma and 32 healthy control subjects were included. All individuals were tested using a triple-staining flow cytometry-based basophil

activation test (BAT) for the potential presence of autoantibodies against FcεRI. Of the 78 asthma patients, 29 (37.2%) had a positive BAT result, indicating that their serum was able to activate basophils, compared with only four (12.5%) of the control group, a statistically significant between-group difference. These data suggest that some asthma patients have aberrant anti-FcεRI autoantibodies, which implies that autoimmunity may be one factor involved in the pathogenesis of intrinsic asthma.

KEY WORDS: ASTHMA; FLOW CYTOMETRY; AUTOANTIBODIES; HIGH-AFFINITY IgE RECEPTOR; CD63 EXPRESSION

Introduction

Asthma is a chronic inflammatory disorder of the airways which has a long history, even being described by Hippocrates.^{1,2} Traditionally, asthma has been regarded as a clinical manifestation of an allergic response to environmental agents, however considerable evidence now suggests that molecular defects in T helper (Th) lymphocyte-mediated immune regulation and multiple immune reactions contribute to the pathogenesis of asthma.^{3–7} The potential

mechanisms other than atopy remain to be clarified. Allergy and autoimmune disease are two potential outcomes of dysregulated immunity; both are characterized by localized inflammation that leads to injury and/or destruction of target tissues.⁸ Until recently, it was generally accepted that the mechanisms that govern these disease processes are quite disparate, however, new discoveries suggest a possible pathogenetic link between allergy and autoimmunity.⁹ Basophil granulocytes and tissue mast cells and their mediators have been found to play a role in the pathogenesis of asthma.^{10,11}

*RS Sun and JF Sui contributed equally to this article.

Human basophils and mast cells (that are positive for the expression of the high-affinity receptor for immunoglobulin E [FcεRI⁺]) can be activated through immunological interaction with the IgE-FcεRI network.^{12,13} Anti-IgE autoantibodies, although occasionally present in normal donors, more frequently occur in a variety of allergic diseases such as chronic urticaria, atopic dermatitis and bronchial asthma, as well as in autoimmune disorders such as rheumatoid arthritis, lupus erythematosus and systemic sclerosis.¹⁴ The flow cytometry-based basophil activation test (BAT), based on the detection of allergen-induced CD63 and CD203c expression, has proved effective in the diagnosis of various IgE-mediated allergies.¹⁵ The aim of the present study was to investigate basophil activation by the functional autoantibodies against FcεRI, as determined by the induction of CD63 expression using the flow cytometry-based BAT, in patients with asthma.

Patients and methods

PATIENTS

Subjects with a cough for more than 3 weeks and who exhibited one or more asthma symptoms and physical examinations compatible with the American Thoracic Society guidelines on the definition of asthma¹⁶ were recruited randomly from the outpatient clinic of Guangzhou Institute of Respiratory Disease, Guangzhou, China, between October 2004 and February 2006. Healthy individuals awaiting elective eye surgery were recruited as controls. One non-allergic adult, whose basophils were screened with antibody directed against FcεRI (Bühlmann Laboratories AG, Allschwil, Switzerland) for > 25% release,¹⁷ was used to provide basophils for the BAT. All subjects gave informed consent and the study was approved by the Ethics Committee of the

First Affiliated Hospital of Guangzhou Medical College.

FLOW CYTOMETRY-BASED BAT

All tests were carried out within 4 h of collection of whole blood from the non-allergic adult donor. The induction of CD63 expression was evaluated as follows.^{18,19} Sodium heparin-anticoagulated peripheral blood aliquots (100 µl) primed with 20 µl of interleukin (IL)-3 containing buffer (10 ng/ml, 10 min, 37 °C) were stimulated at 37 °C for 20 min with 100 µl of serum from either an asthma patient or a healthy control subject. The buffer comprised 0.12 M NaCl, 0.005 M KCl, 0.025 M Tris, pH 7.6. *N*-Formyl-methionyl-leucyl-phenyl-alanine (fMLP 0.4 mmol/l) (Sigma-Aldrich, St Louis, MO, USA), a non-specific cell activator, was used as a positive control and washing buffer (0.01 M phosphate buffered saline including 0.01 M NaH₂PO₄, 0.01 M Na₂HPO₄, pH 7.2 – 7.4) was used as a negative control. After incubation, samples were cooled on ice to stop basophil activation and degranulation. The cells were mixed with staining reagents including fluorescein isothiocyanate (FITC)-conjugated anti-CD63 antibody (Becton Dickinson, Franklin Lakes, NJ, USA), phytoerythrin (PE)-conjugated anti-IgE antibody (Pharmacia, Uppsala, Sweden) and peridinin–chlorophyll–protein complex (PerCP)-conjugated anti-CD45 antibody (Becton Dickinson). After a 20-min incubation on ice in the dark, an erythrocyte lysing solution (Becton Dickinson) was used to lyse the erythrocytes and they were then washed in washing buffer and centrifuged for 10 min at 2500 rpm (desk-top centrifuge; Beckman Instruments, Palo Alto, CA, USA). The leucocyte pellets were resuspended in washing buffer and analysed by fluorescence-activated cell sorting flow cytometry (FC500; Beckman Coulter, Fullerton, CA, USA).

During acquisition, red fluorescence (FL2) and the forward scatter (FSC) and side scatter (SSC) characteristics were employed to gate at least 1000 basophils that expressed a high surface density of IgE. For analysis, a first gate using the FSC/SSC characteristics of lymphocytes was defined. Then, from these cells, basophils were selected as CD45 low/IgE high on an FL3/FL2 dot plot. Within this gate, the percentage of activated basophils, i.e. those co-expressing CD63 (FL1) was measured. For this purpose, quadrant markers were set on the value obtained without stimulation (negative control samples). Percentages of activated basophils were corrected by subtracting spontaneous CD63 expression (negative control) from the values obtained after stimulation with serum from individual patients and control subjects.

STATISTICAL ANALYSIS

Statistical evaluation was performed using the SPSS® software program (SPSS Inc., Chicago, IL, USA). The χ^2 test was used to compare the patient and control groups. A *P*-value < 0.05 was considered significant.

Results

A total of 78 asthma patients and 32 healthy control subjects were included in the study.

MULTICOLOUR FLOW CYTOMETRY STRATEGY

The study used three-colour flow cytometric

BAT protocols that relied on (i) the surface expression characteristics of basophils, i.e. low CD45 and high IgE expression, to identify the total basophil population, and (ii) on the monitoring of the basophil activation marker CD63 to determine the proportion of basophils that had been activated by exposure to patient and control serum. Triple-staining readily identified the total basophil population (high IgE) and localized them within the low CD45 region (Fig. 1).

CD63 EXPRESSION

Table 1 shows the number and proportion of patients and healthy control subjects with CD63⁺ basophils (autoantibodies against FcεRI) following serum challenge of whole blood from the non-allergenic adult donor. Using a cut-off of 8.1% of the level obtained following buffer challenge of whole blood from the non-allergenic adult, serum from 29/78 (37.2%) asthma patients showed CD63⁺ basophils and the mean ± SD proportion of the basophil population that was CD63⁺ was 36.9 ± 8.3%. In contrast, the serum from only 4/32 (12.5%) of the healthy control subjects showed CD63⁺ basophils and the mean ± SD proportion of the basophil population that was CD63⁺ was 26.3 ± 5.6%. The difference in the proportion of asthma patients versus the proportion of healthy control subjects having CD63⁺ basophils was statistically significant (*P* < 0.05).

TABLE 1:
Number and proportion of patients and healthy control subjects with CD63⁺ basophils (autoantibodies against FcεRI) following serum challenge of whole blood from a non-allergenic adult donor

	No. (%) with CD63 ⁺ basophils	χ^2 , <i>P</i> -value
Asthma patients (<i>n</i> = 78)	29 (37.2%)	5.46, <i>P</i> < 0.05
Healthy controls (<i>n</i> = 32)	4 (12.5%)	

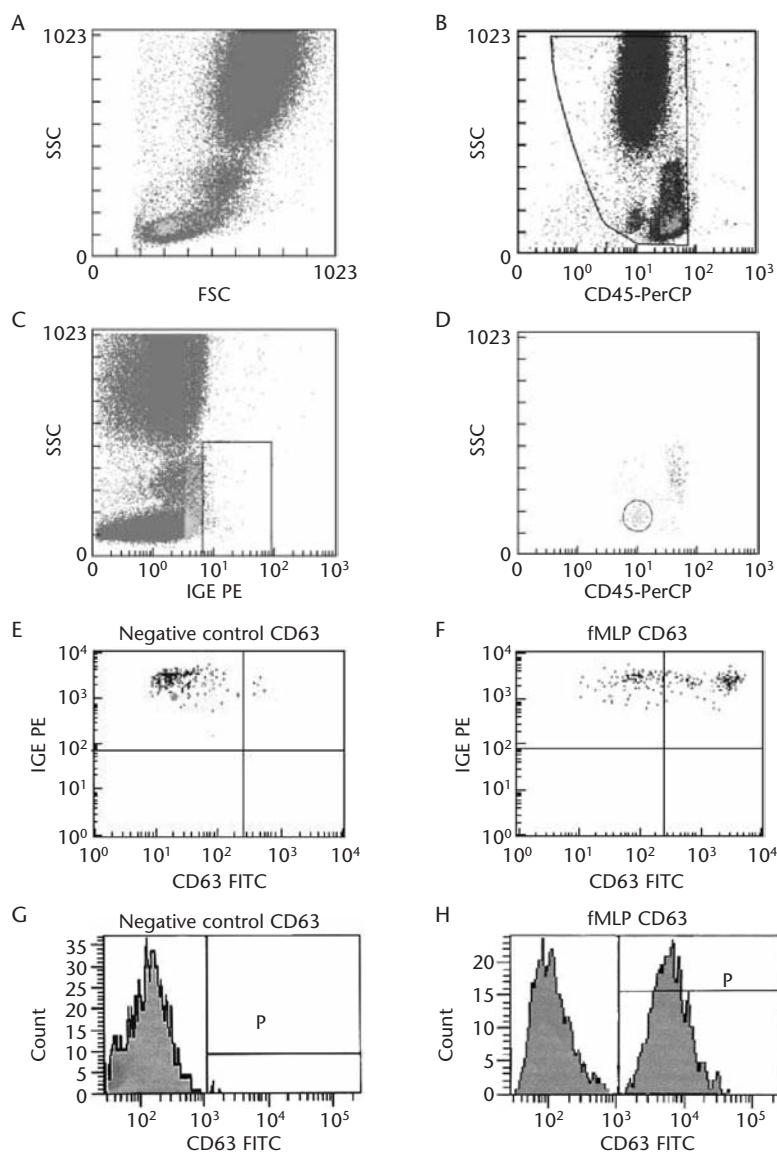


FIGURE 1: Gating protocol to select CD63⁺ basophils using (A) fluorescence (FL), forward scatter (FSC) and side scatter (SSC) characteristics. Whole blood (100 μ l) from a non-allergenic adult was incubated in the presence (positive control) or absence (negative control) of *N*-formyl-methionyl-leucyl-phenylalanine (fMLP). After staining with fluorescein isothiocyanate (FITC)-conjugated anti-CD63 antibody, peridinin-chlorophyll-protein complex (PerCP)-conjugated anti-CD45 antibody and phytoerythrin (PE)-conjugated anti-IgE antibody, the cells were analysed by fluorescence-activated cell sorting flow cytometry. The FL-2/FL-3 plots (B, C, D) identify CD45 low/IgE high basophils and show CD63⁺ basophils (outlined areas). Quadrant markers were then set on the negative control values to eliminate spontaneous levels of CD63 expression to obtain the true level of CD63⁺ basophils (E, F) and this is also shown graphically (G, H)

Discussion

The present study provides further insight into the role of the high-affinity IgE receptor in the pathogenesis of asthma. Flow cytometry-based BAT showed that autoantibodies against FcεRI were observed in significantly more asthma patients than healthy controls (37.2% versus 12.5%, respectively), consistent with the results of our previous research²⁰, which suggests that autoantibodies against FcεRI may be involved in the development of asthma. Asthma mechanisms, apart from atopy, in the development of airway responsiveness and inflammation remain to be clarified, however autoimmune disease has been implicated as a cause of intrinsic asthma.²¹

The high-affinity IgE receptor, FcεRI, is a multimeric surface receptor that is expressed exclusively as a tetramer on rodent cells, but exists as a tetramer or trimer on human cells.²² On mast cells and basophils, FcεRI is essential for IgE-mediated acute allergic reactions.²³ Cross-linking of the IgE-loaded FcεRI by multivalent antigens results in mast cell activation and subsequent release of multiple pro-inflammatory mediators.²⁴ Our research group has previously found anti-FcεRI autoantibodies in some urticaria patients.^{17,25} Considering this finding and that of the present study in asthma patients, allergy and autoimmunity might result from

dysregulation of the immune system. The concomitant presentation of urticaria and asthma, and the potential relationship between them, such as the sharing of common pathogenic mechanisms, are revealed in some cases by looking at the key elements that regulate the immune response in both asthma and autoimmune disease, i.e. mast cells, antibodies, T cells, cytokines and genetic determinants.^{26,27}

It has previously been suggested that autoantibodies against FcεRI or IgE may be involved in the pathogenesis of asthma.^{28–30} The present study's finding that significantly more asthma patients than healthy controls demonstrated positive autoantibodies against FcεRI supports the view that autoimmunity may be involved in the pathogenesis of asthma.

Acknowledgements

This work was supported by the Chinese National 863 Project 2006AA02A121 and an Academician Grant from the Scientific and Technological Committee of Chongqing, China (995691). The authors are grateful to Professor Jason J Chen, Guangzhou Institute of Respiratory Disease (Guangzhou, China) for reviewing the manuscript.

Conflicts of interest

The authors had no conflicts of interest to declare in relation to this article.

- Received for publication 9 July 2008 • Accepted subject to revision 1 August 2008
- Revised accepted 20 October 2008

Copyright © 2008 Field House Publishing LLP

References

- 1 Global Initiative for Asthma: Global strategy for asthma management and prevention. National Institutes of Health (NIH) publication No. 02-3659. Bethesda: NIH, 2002; pp 50–66.
- 2 Keeney EL: The history of asthma from Hippocrates to Meltzer. *J Allergy Clin Immunol* 1964; **35**: 215–226.
- 3 Neurath MF, Finotto S, Glimcher LH: The role of Th1/Th2 polarization in mucosal immunity. *Nat Med* 2002; **8**: 567–573.
- 4 Cohn L, Elias JA, Chupp GL: Asthma: mechanisms of disease persistence and progression. *Annu Rev Immunol* 2004; **22**: 789–815.
- 5 Holloway JW, Beghe B, Holgate ST: The genetic basis of atopic asthma. *Clin Exp Allergy* 1999; **29**: 1023–1032.

- 6 Barnes KC: Evidence for common genetic elements in allergic disease. *J Allergy Clin Immunol* 2000; **106**: 192 – 200.
- 7 Pearce N, Pekkanen J, Beasley R: How much asthma is really attributable to atopy? *Thorax* 1999; **54**: 268 – 272.
- 8 Posnett DN, Yarilin D: Amplification of autoimmune disease by infection. *Arthritis Res Ther* 2005; **7**: 74 – 84.
- 9 Valenta R, Natter S, Seiberler S, *et al*: Isolation of cDNAs coding for IgE autoantigens: a link between atopy and autoimmunity. *Int Arch Allergy Immunol* 1997; **113**: 209 – 212.
- 10 D'Amato G: Role of anti-IgE monoclonal antibody (omalizumab) in the treatment of bronchial asthma and allergic respiratory diseases. *Eur J Pharmacol* 2006; **533**: 302 – 307.
- 11 Denburg JA: Basophils and mast cells in airway inflammation and asthma. *Can Respir J* 1998; **5**: 41A – 44A.
- 12 Qu X, Sada K, Kyo S, *et al*: Negative regulation of FcεRI-mediated mast cell activation by a ubiquitin–protein ligase Cbl-b. *Blood* 2004; **103**: 1779 – 1786.
- 13 Zhu D, Kopley CL, Zhang M, *et al*: Novel human immunoglobulin Fcγ-Fcε bifunctional fusion protein inhibits FcεRI-mediated degranulation. *Nat Med* 2002; **8**: 518 – 521.
- 14 Marone G, Spadaro G, Palumbo C, *et al*: The anti-IgE/anti-FcRIα autoantibody network in allergic and autoimmune diseases. *Clin Exp Allergy* 1999; **29**: 17 – 27.
- 15 Ebo DG, Sainte-Laudy J, Bridts CH, *et al*: Flow-assisted allergy diagnosis: current application and future perspectives. *Allergy* 2006; **61**: 1028 – 1039.
- 16 American Thoracic Society: Lung function testing: selection of reference values and interpretative strategies. *Am Rev Respir Dis* 1991; **144**: 1202 – 1218.
- 17 Sun RS, Liu RQ, Ye QY: Evaluation of histamine-releasing activity in the serum of the patients with chronic idiopathic urticaria. *Acta Acad Med Mil Tert* 2000; **22**: 349 – 350 [in Chinese, English abstract].
- 18 Xu C, Wang X, Jiang L, *et al*: Flow cytometry analysis of basophil degranulation in allergy to dust mite. *Lab Med* 2007; **22**: 245 – 250 [in Chinese, English abstract].
- 19 Ocmant A, Peignois Y, Mulier S, *et al*: Flow cytometry for basophil activation markers: The measurement of CD203c up-regulation is as reliable as CD63 expression in the diagnosis of cat allergy. *J Immunol Methods* 2007; **320**: 40 – 48.
- 20 Sun RS, Chen XH, Liu RQ, *et al*: Autoantibodies to the high-affinity IgE receptor in patients with asthma. *Asian Pac J Allergy Immunol* 2008; **26**: 19 – 22.
- 21 Wiesch DG, Meyers DA, Bleecker ER: Genetics of asthma. *J Allergy Clin Immunol* 1999; **104**: 895 – 901.
- 22 Kinet JP: The high-affinity IgE receptor (FcεRI): from physiology to pathology. *Annu Rev Immunol* 1999; **17**: 931 – 972.
- 23 Honda Z: Fcε- and Fcγ-receptor signaling in diseases. *Springer Semin Immunopathol* 2006; **28**: 365 – 375.
- 24 Kraft S, Rana S, Jouvin MH, *et al*: The role of the FcεRI β-chain in allergic diseases. *Int Arch Allergy Immunol* 2004; **135**: 62 – 72.
- 25 Sun RS, Ran XZ, Cheng TM, *et al*: Detecting anti-FcεRI autoantibodies and its significance. *Sci Technol Rev* 2006; **24**: 59 – 61 [in Chinese, English abstract].
- 26 Larche M: Regulatory T cells in allergy and asthma. *Chest* 2007; **132**: 1007 – 1014.
- 27 Reuter S, Taube C: Mast cells and the development of allergic airway disease. *J Occup Med Toxicol* 2008; **3(suppl 1)**: S2.
- 28 Szczeklik A, Nizankowska E, Serafin A, *et al*: Autoimmune phenomena in bronchial asthma with special reference to aspirin intolerance. *Am J Respir Crit Care Med* 1995; **152**: 1753 – 1756.
- 29 Quintero JM, Burrell RG, Cather CH, *et al*: Detection of antibodies to sinus tissue in chronic bronchial asthma. *Allergy* 1966; **37**: 84 – 89.
- 30 Dinu IV, Roth L: The presence of antilung antibodies in the sera of asthma patients. *Rum Med Rev* 1965; **19**: 26 – 29.

Author's address for correspondence

Professor Xiaohong Chen

The National Key Laboratory of Respiratory Disease, Guangzhou Institute of Respiratory Disease, First Affiliated Hospital of Guangzhou Medical College, Guangzhou 510120, China.

E-mail: sunyueyue@yahoo.com