

IMPORTANCE OF BASOPHILS IN EOSINOPHILIC ASTHMA: THE MURINE COUNTERPART

D. PODDIGHE¹, C.B. MATHIAS³, I. BRAMBILLA², G.L. MARSEGLIA² and H.C. OETTGEN^{4,5}

¹Department of Medicine, School of Medicine, Nazarbayev University, Astana, Kazakhstan;

²Department of Pediatrics, Università degli Studi, Pavia, Italy; ³Department of Pharmaceutical and Administrative Sciences, College of Pharmacy, Western New England University, Springfield, MA, USA; ⁴Department of Medicine, Boston Children's Hospital, Boston, MA, USA; ⁵Department of Pediatrics, Harvard Medical School, Boston, MA, USA

Received December 19, 2017 – Accepted February 19, 2018

Several experimental studies in mice showed that basophils participate in the initiation of Th2 adaptive immune response, in addition to the effector phase. However, the role of basophils in allergic airway inflammation is less clear. The aim of this experiment was to assess the importance of basophils in recruiting inflammatory cells and, in particular, eosinophils in a murine model of asthma induced by *Aspergillus fumigatus* allergens. Additionally, bronchial reactivity was evaluated. Basophil depletion resulted in a reduction of inflammatory cells in the airways and eosinophil recruitment was significantly impaired. Also bronchial reactivity seemed to be impaired in basophil-depleted mice, but the result was not statistically significant. These preliminary data seem to support a role of basophils in the local eosinophilic response of allergic asthma.

To the Editor,

Allergic asthma is a chronic inflammatory process arising from a dysregulation of the immune response to some ubiquitous environmental allergens. Cytokines, such as interleukin (IL)-4, IL-5, IL-13, produced by CD4⁺ T cells polarized toward Th2 functional phenotype and through activated mast cells, lead to IgE production and eosinophilic pulmonary inflammation associated to airway hyper-responsiveness, which are the main pathophysiological features of allergic asthma (1).

Initial mechanisms responsible for Th2 differentiation after contact with air allergens have not yet been completely elucidated. Local cytokine production along with antigenic activation of naïve T CD4⁺ cells is known to be an important factor

to commit them toward one of several functional T helper subsets. In allergic diseases, early IL-4 production is supposed to be critical for Th2 differentiation, and the innate immune system has been demonstrated to play a fundamental role in this regard. Recently, the idea that basophils may be an important starting source of lymphocyte-priming IL-4 has been gaining ground progressively (2-3).

Despite their small number, basophils have been shown to contribute to the effector phase of host response to helminthes, chronic IgE-mediated skin reactions and acute IgG-mediated anaphylaxis; moreover, recent studies hypothesized a role of basophils in some autoimmune diseases. In general, novel mouse models provide additional evidence supporting an essential role of basophils

Key words: basophils, asthma, mouse, Aspergillus fumigatus, eosinophils, bronchial reactivity

Mailing address:

Dr. Dimitri Poddighe, M.D.

Department of Medicine,

School of Medicine, Nazarbayev University,

010000 Astana, Kazakhstan

Tel.: +7 347 7241851

e-mail: dimitri.poddighe@nu.edu.kz

0393-974X (2018)

Copyright © by BIOLIFE, s.a.s.

This publication and/or article is for individual use only and may not be further reproduced without written permission from the copyright holder.

Unauthorized reproduction may result in financial and other penalties

DISCLOSURE: ALL AUTHORS REPORT NO CONFLICTS OF INTEREST RELEVANT TO THIS ARTICLE.

in triggering Th2 responses in allergy (4-7). A few years ago, we carried out a study on a murine model of allergic asthma induced by *Aspergillus fumigatus* (*Af*) allergens. Our previous findings from such a mouse model of eosinophilic asthma suggested that basophils promote the development of Th2 lymphocytes, as their depletion resulted in reducing the percentage of Th2-lymphocytes at several sites, as well as the related cytokine and IgE immunoglobulin production. (8)

As a clinical counterpart, an increased number of basophils in lungs and airways of asthmatic patients has been described in several studies, but those cells are mainly implicated in eosinophilic asthma. Recently, Brooks et al. assessed the number of basophils in the sputum of patients with allergic asthma and found an increase of basophils, recovering the highest numbers of basophils in patients showing an eosinophilic endotype of asthma (9). Thus, in the light of recent advances in the knowledge of the biological importance of basophils, here we report some preliminary data collected in the context of experimental studies on basophil-depleted mice with allergic asthma induced by *Aspergillus fumigatus* (*Af*) allergens, which further support the role of basophils in the recruitment of eosinophils in the inflamed respiratory mucosa and, as a consequence, for bronchial hyper-reactivity.

MATERIALS AND METHODS

Study design

A 50 μ l sterile aqueous extract of *Af* was applied intranasally (*i.n.*) to BALB/c mice, according to a prolonged sensitization protocol, lasting 3 weeks (3w), as shown in Fig. 1 (10); the control group was treated with intra-nasal sterile saline solution (50 μ l). Mice depleted of basophils received the intra-peritoneal (*i.p.*) administration of MAR-1 anti-Fc ϵ RI monoclonal antibody (10 μ g/day for 3 days/week). Each experimental group included five mice. After the sensitization protocol, all mice underwent whole body plethysmography (through BUXCO equipment), in order to evaluate the bronchial reactivity to nebulized methacholine administered at progressively increasing concentrations (from 0 mg/ml up to 333 mg/ml). After this procedure, mice were sacrificed and a bronchoalveolar

lavage (BAL) was performed, in order to count the inflammatory cells inside.

Mice and reagents

A sterile aqueous extract of *Aspergillus fumigatus* (1:10 w/v at 87.000 PNU/ml) was obtained from Greer Laboratories. BALB/c mice were purchased from Taconic Farms. Mice were housed in a specific pathogen-free environment and were 8-10 weeks old. All experiments were conducted in accordance with the Institutional Animal Care and Use Committee policies and procedures of the Children's Hospital Boston.

Collection and analysis of BAL specimen

For bronchoalveolar lavage (BAL), 1 ml of PBS was infused into the trachea and then retrieved. BAL fluid was cytocentrifugated onto slides and differential cellular analysis was performed by light microscopy, after staining with Wright-Giemsa (Richard-Allan Scientific).

Methacholine bronchial reactivity test

The whole-body single-chambered plethysmograph (freely moving animal) was purchased from BUXCO Electronics, New York, USA. Data were acquired in conscious mice using a BUXCO LS14 Biosystem Plethysmograph logging analyzer. Penh (PEF/PIF $\times$ Pause) was used as a main parameter to track the bronchial response to allergen challenge (11).

Statistical analysis

Differences in experimental parameters among the experimental groups were examined for significance with GraphPad Prism® software using ANOVA test, and values are presented as means $\pm$ standard error of the mean (SEM).

RESULTS

BAL cellularity

After sacrifice, the mice received bronchoalveolar lavage (BAL) in order to count the inflammatory cells from lower airways.

As shown in Fig. 2A, we observed a significant increase of cells recovered by BAL in mice sensitized with *Af* suspension compared to mice receiving sterile saline solution ($638 \pm 52.1 \times 10^3/\text{ml}$ vs $52.5 \pm 8.5 \times 10^3/\text{ml}$

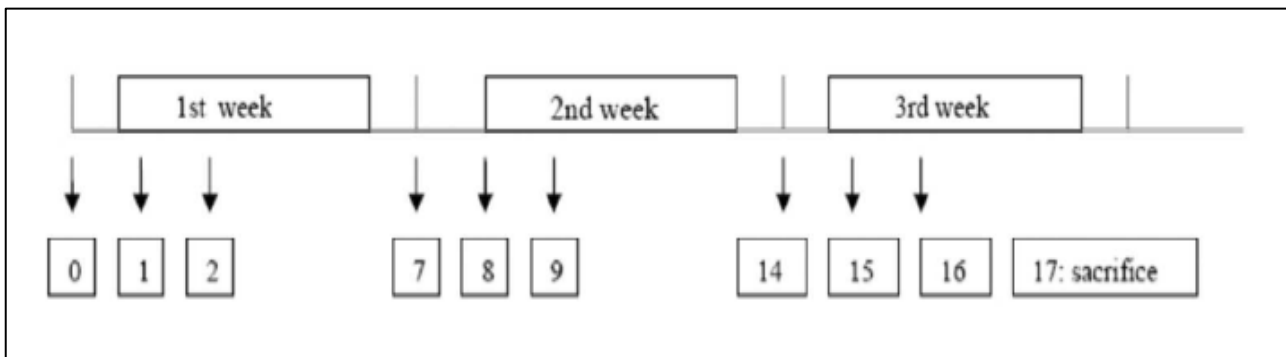


Fig. 1. Sensitization protocol with *Aspergillus fumigatus* allergens (the arrows indicate days when the extract was administered intra-nasally).

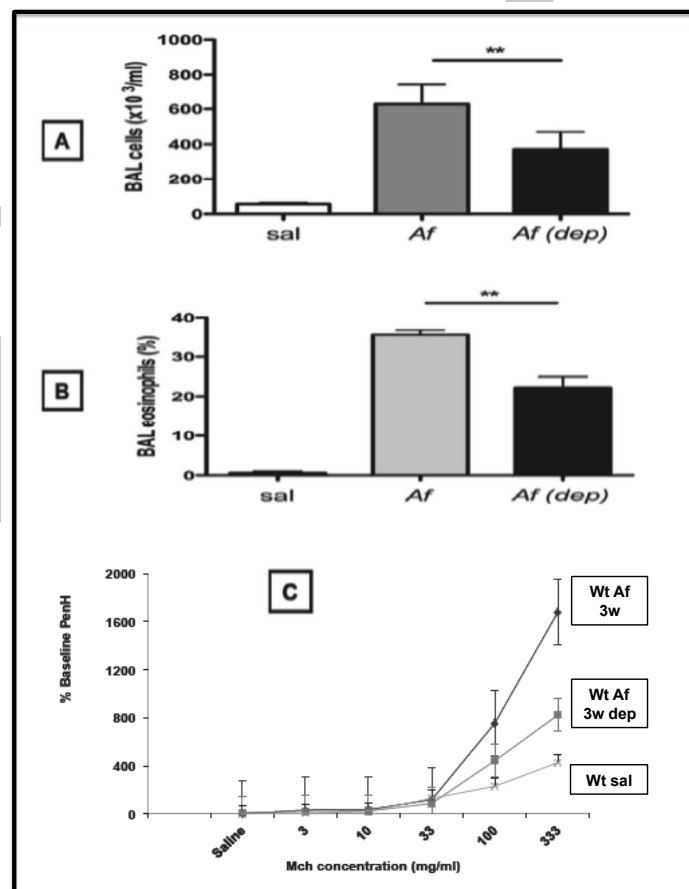


Fig. 2. Summary of experimental data. **A)** BAL cellularity in the three groups of mice: wild-type mice receiving saline solution [sal], wild-type mice sensitized with *Aspergillus fumigatus* allergens [Af] and basophil-depleted wild-type mice sensitized with *Aspergillus fumigatus* allergens [Af (dep)]. **B)** Percentage of eosinophils in BAL cells in the three experimental groups. **C)** Curves of bronchial resistances after methacholine nebulization for all three groups: wild-type mice receiving saline solution [WT sal], wild-type mice sensitized with *Aspergillus fumigatus* allergens [WT Af 3w] and basophil-depleted wild-type mice sensitized with *Aspergillus fumigatus* allergens [WT Af 3w dep].

in saline-treated mice, $p < 0.001$); importantly, basophil-depleted and *Af* sensitized mice showed a significant reduction of total cellularity in BAL ($372 \pm 43.75 \times 10^3/\text{ml}$, $p < 0.01$) compared to the correspondent group untreated with MAR-1 anti-Fc ϵ RI monoclonal antibody.

Moreover, BAL eosinophils were specifically counted. Whereas the inflammatory infiltrate was characterized by many eosinophils in normal mice sensitized with *Af* ($243 \pm 28.9 \times 10^3/\text{ml}$), their number was significantly lower in the study group depleted of basophils ($81.8 \pm 10.5 \times 10^3/\text{ml}$, $p < 0.001$). No eosinophil increase was seen in mice treated with saline solution. In order to assess the specific difference in eosinophil recruitment, we expressed these data in percentage of the total cellularity (Fig. 2B). Again, the percentage of eosinophils ($35.8 \pm 1.1\%$ vs $22.2 \pm 2.75\%$, $p < 0.01$) resulted to be significantly lower in mice depleted of basophils.

Bronchial reactivity

The curves expressing bronchial resistance (obtained through the relative increase of the measured PenH at a specific concentration of nebulized methacholine, expressed in percentage of the correspondent basal value) for each experimental group of mice are shown in Fig. 2C. At the two highest concentrations of nebulized methacholine, namely 100 mg/ml and 333 mg/ml, differences among the three curves seem to develop: in particular, the bronchial resistance of basophil-depleted mice resulted to be less increased than normal mice, after the protocol of *Af* sensitization, but such a difference did not reach any statistical significance.

DISCUSSION

Basophils have been implicated as amplifier/effector cells of allergic inflammation and Th2 responses, through IgE binding. Indeed, a number of studies appreciated the effector role of basophils in anaphylaxis and allergic skin inflammation. More recently, skin inflammation elicited by means of passive immunization with hapten-specific IgE, followed by intradermal challenge, demonstrated the implication of basophils also in the induction of IgE-mediated allergic inflammation. Subsequent investigations, using genetic strategies to get mouse models depleted of basophils, confirmed a block

of the induction of IgE-driven skin inflammation. Finally, an experimental study reported that the induction of food allergy by means of epicutaneous antigen application is dependent on IgE antibodies and basophils (12-13).

Unfortunately, the role of basophils in allergic airway inflammation is less defined. However, basophil depletion in murine models of OVA-induced allergic airway inflammation supported their role in the recruitment of Th2 cells in the airways. (14) We also showed an impaired development of IL-4-producing Th2 lymphocytes and IgE responses after basophil depletion in our murine model of allergic asthma induced by *Aspergillus fumigatus* allergens. (8) Interestingly, as a human counterpart, Boita et al. demonstrated that *Aspergillus fumigatus* stimulation resulted to upregulate BAFF expression on the basophils of severe asthmatic patients and those affected with bronchopulmonary aspergillosis, suggesting that basophils may contribute to the Th2 adaptive immune response and, specifically, to the polyclonal production of IgE observed in these patients. (15)

Moreover, basophils resulted to be increased in lungs of asthmatics and, importantly, were detected in the lungs of fatal asthma patients, suggesting an important role in most severe forms (16). The recent article by Brooks et al. supported a prominent role of basophils in eosinophilic asthma, where airway inflammation is typically Th2-driven: In that study, the authors assessed the number of basophils in the sputum of patients with allergic asthma compared to healthy controls; they found an increase of basophils in all asthmatic patients, recovering the highest numbers of basophils in patients showing an eosinophilic asthma. However, they were not able to find a clear correlation with respiratory function tests and, probably, this lack of statistical significance could be related to the small number of patients included in each subgroup of asthma (9). In the light of these human findings, our preliminary data may represent the murine counterpart. In our murine model of allergic asthma induced by *Aspergillus fumigatus* allergens, mice subjected to repeated inhalation of *Af* displayed a vigorous IgE response accompanied by a robust expansion of mast cells in

the bronchi, which was associated with a significant recruitment of eosinophils also in the airways. Thus, such a model could be assimilated to human eosinophilic asthma. After describing that basophil depletion resulted in a significant impairment of Th2 adaptive immune response, here we assessed the bronchial inflammatory response and the bronchial reactivity to methacholine challenge. The cellularity of BAL resulted reduced in basophil-depleted mice and, importantly, that primarily compromised the eosinophilic component. Thus, our experimental data enriched this pathophysiological picture by showing that Th2 reduction, induced by basophil depletion, may impair the effector phase of the allergic immune response in the airways and the bronchial reactivity.

In conclusion, our data from a murine experimental model support the clinical observations of the importance of basophils in those endotypes of human asthma characterized by eosinophil infiltration, according to few available human studies. These findings might be an interesting extension of our previous studies, but further research is needed to clarify the biological relevance and mechanisms, which are probably different among different forms of asthma, both in humans and animal models. Importantly, if this initial evidence could be confirmed by future research, basophils might represent an additional target for the therapy of allergic and eosinophilic endotypes of asthma.

REFERENCES

1. Umetsu DT, Dekruyff RH. Immune dysregulation in asthma. *Curr Opin Immunol* 2006; 18:727-32.
2. Paul WE, Zhu J. How are T(H)2-type immune responses initiated and amplified? *Nat Rev Immunol* 2010; 10:225-35.
3. Thirion D, Morianos I, Xanthou G, Samitas K. Innate immunity as the orchestrator of allergic airway inflammation and resolution in asthma. *Int Immunopharmacol* 2017; 48:43-54.
4. Obata K, Mukai K, Tsujimura Y, et al. Basophils are essential initiators of a novel type of chronic allergic inflammation. *Blood* 2007; 110:913-20.
5. Tsujimura Y, Obata K, Mukai K, et al. Basophils play a pivotal role in immunoglobulin-G-mediated but not immunoglobulin-E-mediated systemic anaphylaxis. *Immunity* 2008; 28:581-89.
6. Poddighe D, Marseglia GL. Commentary: Basophil activation-dependent autoantibody and interleukin-17 production exacerbate systemic lupus erythematosus. *Front Immunol* 2017; 8:787.
7. Schwartz C, Eberle JU, Voehringer D. Basophils in inflammation. *Eur J Pharmacol* 2016; 778:90-95.
8. Poddighe D, Mathias CB, Freyschmidt EJ, Kombe D, Caplan B, Marseglia GL, Oettgen HC. Basophils are rapidly mobilized following initial aeroallergen encounter in naïve mice and provide a priming source of IL-4 in adaptive immune responses. *J Biol Regul Homeost Agents* 2014; 28:91-103.
9. Brooks CR, van Dalen CJ, Hermans IF, Gibson PG, Simpson JL, Douwes J. Sputum basophils are increased in eosinophilic asthma compared with non-eosinophilic asthma phenotypes. *Allergy* 2017; 72(10):1583-86.
10. Mathias CB, Freyschmidt EJ, Caplan B, et al. IgE influences the number and function of mature mast cells, but not progenitor recruitment in allergic pulmonary inflammation. *J Immunol* 2009; 182:2416-24.
11. Lomask M. Further exploration of the Penh parameter. *Exp Toxicol Pathol* 2006; 57:13-20.
12. Karasuyama H, Yamanishi Y. Basophils have emerged as a key player in immunity. *Curr Opin Immunol* 2014; 31:1-7.
13. Oettgen HC. Fifty years later: Emerging functions of IgE antibodies in host defense, immune regulation, and allergic diseases. *J Allergy Clin Immunol* 2016; 137:631-45.
14. Zhong W, Wu W, Zhang Y, et al. Basophils as a primary inducer of the T helper type 2 immunity in ovalbumin-induced allergic airway inflammation. *Immunology* 2014; 142:202-15.
15. Boita M, Heffler E, Pizzimenti S, et al. Regulation of B-cell-activating factor expression on the basophil membrane of allergic patients. *Int Arch Allergy Immunol* 2015; 166:208-12.
16. Marseglia GL, Caimmi S, Marseglia A, et al. Rhinosinusitis and asthma. *Int J Immunopathol Pharmacol* 2010; 23(S):29-31.