

Activation of basophils by the double-stranded RNA poly(A:U) exacerbates allergic inflammation

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Abstract

Background: It is commonly acknowledged that asthma is exacerbated by viral infections. On the other hand, basophil infiltration of lung tissues has been evidenced postmortem in cases of fatal disease, raising the question of a possible link between these two observations.

Objectives: Herein, we addressed the relationship between asthma exacerbation by viral infection and basophil activation and expansion by investigating how stimulation with the dsRNA polyadenylic/polyuridylic acid [poly(A:U)] affected basophil activities and recruitment in an allergic airway inflammation model.

Methods: The effect of dsRNA on basophils was assessed by measuring the cytokine levels produced upon stimulation. We used an OVA-induced experimental model of allergic asthma. Airway hyperreactivity, recruitment of infiltrating cells, and cytokine production were determined in the lung of mice having received poly(A:U), as compared with untreated controls. The exacerbating effect of basophils was assessed both by adoptive transfer of poly(A:U)-treated basophils and by their *in vivo* depletion with Ba103 antibody.

Results: We found that *in vitro* treatment with poly(A:U) increased basophil functions by inducing T_H2-type cytokine and histamine production, whereas *in vivo* treatment increased peripheral basophil recruitment. Furthermore, we provide the first demonstration for increased infiltration of basophils in the lung of mice suffering from airway inflammation. In this model, disease symptoms were clearly exacerbated upon adoptive transfer of basophils exposed to poly(A:U), relative to their unstimulated counterpart. Conversely, *in vivo* basophil depletion alleviated disease syndromes, thus validating the transfer data.

Conclusions: Our findings provide the first evidence for airway inflammation exacerbation by basophils following dsRNA stimulation.

Basophils are the least abundant granulocytes, representing less than 1% of peripheral blood leukocytes. For this reason, together with their poor phenotypic and morphological characterization and lack of animal models, their specific physiological functions during the immune response have been ignored until

recently. Even though mast cells and basophils are both considered primary effector cells of bronchial asthma, there is compelling immunological, biochemical, and pharmacological evidence that they exert distinct functions at the onset and progression of the asthma phenotype (1). Basophils play key roles in the development of acute and chronic allergic responses, protective immunity against ecto- and endoparasites, and regulation of acquired immunity, including the augmentation of humoral memory responses and the initiation of T_H2 differentiation. Basophils are indeed major early producers of IL-4 and IL-13, which are critical for setting off and maintaining allergic responses (2). Moreover, in agreement with their putative effector

Abbreviations

BALF, Bronchoalveolar lavage fluid; BM, Bone marrow; BMDb, Bone marrow-derived basophils; dsRNA, Double-stranded ribonucleic acid; Mch, Methacholine; poly(A:U), Polyadenylic/polyuridylic acid.

functions, they are clearly associated with fatal asthma (3, 4) and detected in nasal lavage fluid after allergen challenge of patients with allergic rhinitis (5). This notion is further supported by the correlation between gain-of-function mutations of the IL-4R and disease exacerbation (6).

Murine basophils are most frequent in the bone marrow where they originate and most likely complete their maturation, conversely to mast cells, which differentiate in the periphery (7). They constitute an excellent source of IL-4, IL-6, and histamine, in response to growth factors like IL-3 and GM-CSF or calcium ionophore, and IgE (8, 9). Through their activation by IL-33 and thymic stromal lymphopoietin (TSLP), they participate together with DCs in the initiation and amplification of T_H2 -type immune responses (10–12). Recent studies have shown that basophil activation is not only promoted by antigen-specific IgE crosslinking, but can also be caused in nonsensitized individuals by parasitic antigens, lectins, and viral superantigens, either by direct crosslinking of the FcεR or by binding to nonspecific IgE antibodies (13). Furthermore, novel IgE- and FcεR-independent routes of stimulation have been reported, consistent with the assumed regulatory functions of basophils in innate immunity (14). In further support of this notion, several recent studies provide evidence that they can also act as antigen-presenting cells (15–17), in particular in the case of an allergen-induced T_H2 response.

Viral respiratory infections, especially those induced by rhinovirus and respiratory syncytial virus, are the most common and important cause of an acute asthma exacerbation in both adults and children and represent a global health burden (18, 19). An increasing body of evidence supports the hypothesis that these infections cause more morbidity in asthmatic than in healthy individuals. Understanding the mechanisms responsible for virus-induced airway inflammation in asthmatic subjects might offer significant opportunities for improved disease management. Viruses generate replication-mediated dsRNA in infected cells, which serve as a ligand for pattern recognition receptors. We postulated that this type of interaction might lead to increased T_H2 cytokine production by basophils accounting for their adverse effect in allergic asthma. To verify this hypothesis, we tested the effect of dsRNA on typical basophil activities, using purified populations derived from bone marrow cultures. We found that double-stranded polyadenylic/polyuridylic acid [poly(A:U)] activated basophils both *in vitro* and *in vivo* to produce histamine and T_H2 cytokines, such as IL-4, IL-6, and IL-13. We further demonstrate the direct implication of basophils in allergic inflammation exacerbation, by means of either adoptive transfer or depletion with a specific antibody, and assess the physiological relevance of poly(A:U) in this model by showing the aggravation of disease hallmarks when basophils were exposed to the dsRNA before transfer.

Materials and methods

Mice, cytokines, and reagents

This study was performed with male and female 7- to 9-wk-old NOD mice bred in our animal facility. C57BL/6J mice were

purchased from CERJ. $J\alpha18^{-/-}$ mice were originally provided by Dr. T. Taniguchi and bred in our animal facility under specific pathogen-free conditions, as were NOD mice.

Cytokines, antibodies, and other reagents

Murine recombinant IL-3 was purchased from R&D Systems. The following appropriately labeled antibodies were used: anti-mouse CD117 (2B8), CD49b (Hmα2), FcεRIα (MAR-1), CD69 (H1.2F3), and isotype controls (BD Pharmingen). The poly(A:U) used was purchased from Sigma-Aldrich, Midland, or prepared in the laboratory using polyA (Fluka/Sigma-Aldrich) and polyU (MP Biomedical) as described before (20). RNase A was purchased from Sigma-Aldrich.

Sorting of BM-derived basophils and flow cytometry

Basophil-enriched populations were generated from total BM cells cultured for 7–9 days with IL-3, as described before (Schneider et al., 2005). This population contained on average 25–40% basophils defined as c-kit⁺FcεRIα⁺CD49b⁺ cells. To avoid activation by FcεR staining, they were electronically sorted as c-kit⁺CD49b⁺ cells using a FACSaria cell sorter (BD). Purity was >98% upon reanalysis of aliquots stained with anti-FcεRIα mAb. After FcγR blockade by CD16/32, basophils were stained in different organs with CD49b-APC, c-kit-FITC, and FcεRIα-PE mAbs and analyzed in a FACSCanto™ flow cytometer using FACSDiva software (BD). Basophils were also sorted after 6 days of culture and incubated at a concentration of 10^6 per millilitre in the presence of 10 ng/ml IL-3 or 100 μg/ml of poly(A:U) in 24-well plates. Viable cells were counted daily using trypan blue exclusion.

Cell cultures

For cytokine and histamine assays, cells from bone marrow, liver, lung, and sorted basophils were recovered as previously described. The cells were suspended in MEM (Invitrogen) supplemented with 10% horse serum (Invitrogen) instead of FCS, which contains histaminase (21). They were adjusted to a final concentration of 10^7 and 2.5×10^6 of liver and bone marrow cells per millilitre, respectively, and exposed for 24 h to different stimuli, as stated in Results, relative to medium controls. In some cases, poly(A:U) was pretreated with RNase A (100 μg/ml) for 5 h at 37°C and 10^{-6} M. Supernatants were collected and stored at –20°C for cytokine and histamine assays.

Cytokine and histamine assays

IL-4, IL-5, IL-6, IL-13, eotaxin, and TARC were quantified in culture supernatants, BALF, and liver using ELISA kits from R&D according to the manufacturer's instructions. Histamine concentrations in cell supernatants were currently determined by an automated continuous-flow fluorometric technique (lower limit of sensitivity: 50 pg/ml), as previously described (22).

In vivo treatment

Female C57BL/6J or NOD mice received daily *i.p.* injections of 100 µg of poly(A:U) or NaCl for 5 days. 24 h after the last injection, they were anesthetized with ketamine (35 mg/kg, Sigma-Aldrich) before collecting blood from the retro-orbital sinus. After killing, basophils were identified among mononuclear cells from different organs upon staining with CD49b-APC and FcεRIαPE mAbs.

Experimental allergic inflammation

8- to 10-week-old male NOD mice with normal glycosuria were sensitized by *i.p.* injections of 100 mg OVA on days 0, 2, and 4. Thereafter, from day 10 (D 10) to D15, they received a daily treatment with aerosolized OVA at a concentration of 20 mg/ml or NaCl for 20 min, using an ultrasonic nebulizer (Ultra-Neb99). 1 h later, they were given 50 mg poly(A:U) or NaCl *i.n.*

Adoptive transfer or depletion of basophils

Jα18^{-/-} mice immunized according to the above protocol received 10⁶ bone marrow-derived sorted basophils after a 2-h exposure to poly(A:U) or culture medium alone by *i.v.* injection 1 h before the first OVA aerosol challenge (six mice per group). For basophil depletion, immunized male NOD mice were injected *i.p.* with 5 µg of Ba103 antibody per mouse twice a day from D7 to D9. Phenotypic and functional analyses of basophils were performed after killing on D16.

Statistics

All data are expressed as means ± SEM. Statistical significance (**P* < 0.05; ***P* < 0.01; ****P* < 0.005) was established by Student's *t*-test, using GraphPad Prism5 software.

Results

Poly(A:U) targets basophils by inducing basophil recruitment and activation

Knowing that viral infections can exacerbate the pathology in asthmatic patients (18, 19), we postulated that poly(A:U) recognized as a dsRNA pathogen might exert a similar effect through its ability to activate and recruit basophils to the periphery. Basophils are present at very low levels in mice outside the bone marrow. We examined whether poly(A:U) could promote their peripheral recruitment following five daily *i.p.* injections at a dose of 100 µg per mouse. On day 6, we analyzed CD49b⁺FcεRIα⁺ cells in different organs and found increased frequencies among mononuclear cells recovered from both liver and blood (Fig. 1A). The effect was particularly striking in the liver where the percentage of basophils rose from 0.1 to 1%, resulting in enhanced responsiveness to IL-3 in terms of IL-4 and IL-6 production (Fig. 1B). The higher proportion of basophils was associated with an increase in absolute basophil counts

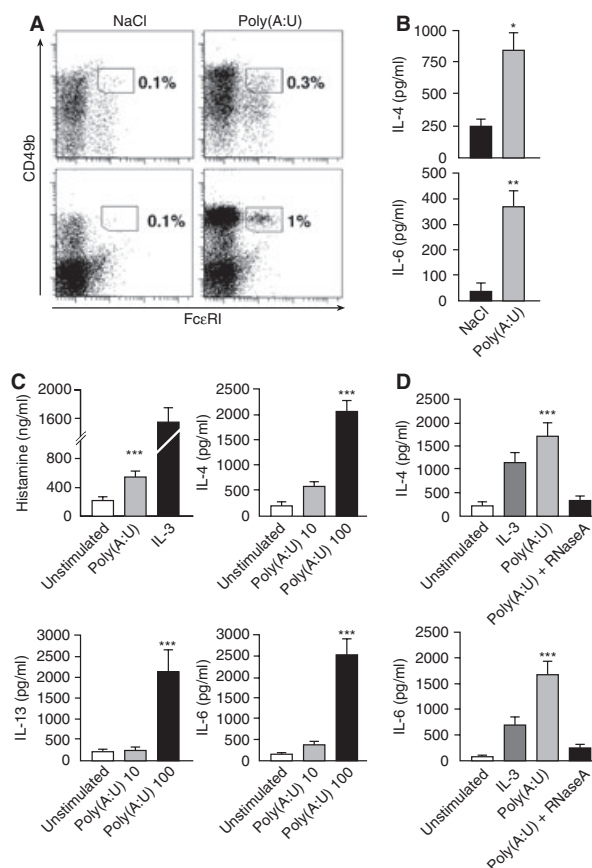


Figure 1 The dsRNA poly(A:U) targets basophils *in vitro* and *in vivo*. (A) Poly(A:U)-induced basophil recruitment to the periphery. NOD mice received daily *i.p.* injections of 100 µg of poly(A:U) or NaCl for five consecutive days. 24 h later, basophils were analyzed in blood (upper panels) and liver (lower panels). Representative flow cytometric profiles of CD49b⁺FcεRIα⁺ basophils in the blood and the liver of treated mice. Numbers in dot blots indicate the percentage of gated basophils. Representative of two independent experiments are shown. (B) Functional activation of hepatic basophils in response to poly(A:U). Mononuclear liver cells (10⁷/ml) from NOD mice having received poly(A:U) or saline as stated above were incubated with IL-3 for 24 h before measuring IL-4 and IL-6 production. Significant changes between PBS and poly(A:U)-treated mice (*n* = 5 mice per group); ***P* ≤ 0.01; **P* ≤ 0.05. (C) Poly(A:U)-induced cytokine and histamine production. 10⁶ sorted basophils/ml were incubated with or without poly(A:U) (100 µg/ml) or IL-3 (10 ng/ml) for 24 h, and histamine was measured in supernatants by fluorometric detection. Data are means ± SEM. Each histogram combines the results of at least four independent experiments. (D) RNA-specific effect of poly(A:U). BMDM were incubated with 100 µg/ml of poly(A:U) pretreated or not with RNase A. IL-4 production was measured in supernatants after a 24-h incubation, as compared with controls set up in culture medium or stimulated with IL-3. Data are means ± SEM; each histogram combines the results of at least five independent experiments.

in the liver from $1.963 \pm 0.46 \times 10^3$ after NaCl treatment to $44.06 \pm 0.93 \times 10^3$ following dsRNA stimulation, while no consistent increase in basophil counts occurred in the

spleen and in the bone marrow (data not shown). We further examined the cytokine production by basophils purified from enriched populations generated during 7–9 days of culture in the presence of IL-3, followed by electronic sorting of CD49b⁺c-kit⁺ cells. These cells responded to poly(A:U) in a dose-dependent manner by secreting IL-4, IL-6, and IL-13 (Fig. 1C). Cytokine production failed to occur after treatment with RNase A, as illustrated by IL-4 production in Fig. 1D, which proves its RNA specificity. RNase A had no effect on its own and did not modify IL-3-induced histamine and cytokine production (data not shown).

Poly(A:U) aggravates allergic asthma through activation and recruitment of basophils

Basophils are believed to carry out important effector functions during allergic inflammation, but have not been detected so far in bronchoalveolar fluid (BALF) in murine experimental allergic asthma. We used NOD mice, which develop a more pronounced allergic response than the C57BL/6 strain (23), to assess whether the incidence and activity of basophils increased during disease progression. To mimic viral respiratory tract infections, poly(A:U) was given *i.n.* to immunized mice 1 h after the OVA challenge from days 10 to 15. In these conditions, we detected a significant increase in the incidence of pulmonary basophils that has not been reported in this model before (Fig. 2A). It occurred already in mice challenged with OVA alone and increased substantially after administration of poly(A:U), concurrently with a rise in the hyperreactivity (AHR), an increase in infiltrating cells, such as eosinophils in BALF, and an enhanced production of the pro-T_H2 cytokines IL-4, IL-5, IL-6, and IL-13, along with the chemokine eotaxin (Fig. 2B,C,D).

To establish that basophils increased disease symptoms directly, we took advantage of an adoptive transfer model with purified basophils. We optimized this procedure using a strain known for its moderate asthma response, namely iNKT cell-deficient J α 18^{-/-} mice. Interestingly, the incidence of basophils in the bone marrow of these mice was also lower than in their wild type counterpart ($0.28 \pm 0.01\%$ vs $0.6 \pm 0.04\%$, respectively). Basophils were purified from enriched populations generated during 7–9 days of culture in the presence of IL-3, followed by electronic sorting of CD49b⁺c-kit⁺ cells, which yielded a >95% pure population upon reanalysis (Fig. 3A). Sorted cells were treated for 2 h with or without 100 μ g/ml of poly(A:U) prior to retro-orbital injection, 1 h before the first aerosol challenge with OVA of mice previously immunized on days 0, 2, and 4 with 100 μ g of OVA. The treatment with 20 mg OVA in aerosol was continued from day 10 to day 15, followed by killing on day 16. As shown in Fig. 3B,C, these mice, which are known for mounting a poor T_H2 response, develop modest asthma symptoms in terms of eosinophil infiltration and secretion of eotaxin and TARC, which become more severe in the group of mice having received unstimulated basophils. Interestingly, the inflammatory responses were further aggravated after exposure of basophils to poly(A:U) prior to injection (Fig. 3B,C).

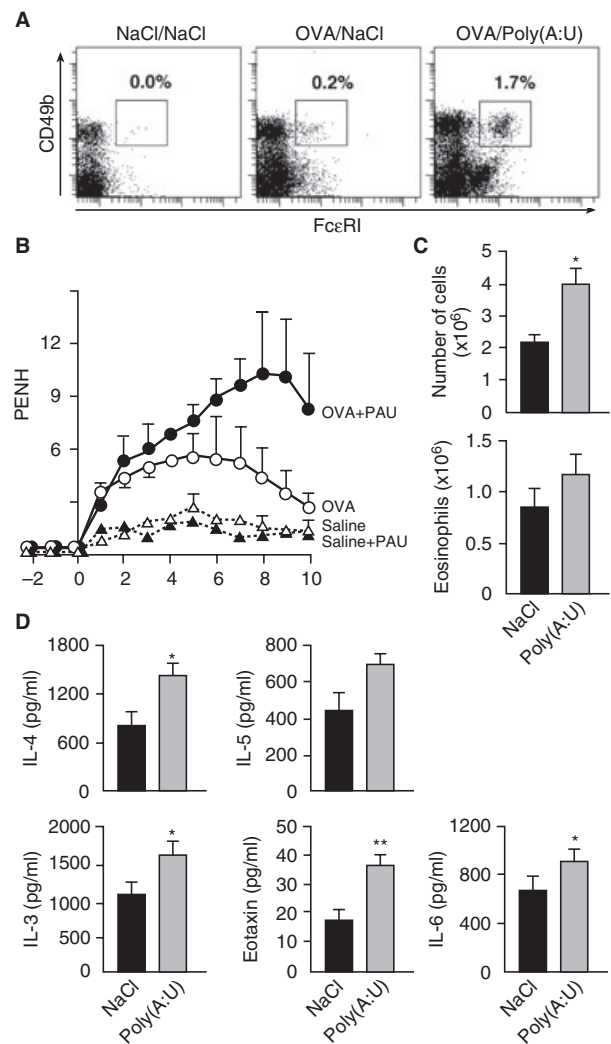


Figure 2 Increased basophil levels coincide with asthma exacerbation. (A) Increased basophil recruitment to the lung of immunized mice having received poly(A:U). NOD mice were immunized according to the protocol described in Materials and methods. One group was challenged with NaCl followed by *i.n.* NaCl inoculation, and a second group was challenged with OVA followed by *i.n.* administration of either NaCl or 50 μ g of poly(A:U) 1 h after OVA aerosol challenge from D10–D14. Basophil recruitment was analyzed in the lungs. (B) Mice treated with poly(A:U) showed an increased AHR as compared to control mice. AHR to Mch was measured 24 h after the last challenge. (C, D) Mice treated with poly(A:U) showed an increased eosinophilia in BALF and IL-4 and eotaxin production as compared to control mice. These experiments were performed twice using 4–6 mice per group (each value represents one mouse). One representative experiment is shown.

Basophil depletion attenuates allergic asthma syndromes

We chose the inverse strategy, namely basophil depletion, to validate the transfer data. To this end, we injected the monoclonal antibody Ba103 (24) from days 7 to 9, twice a day. The treatment was very effective as it decreased

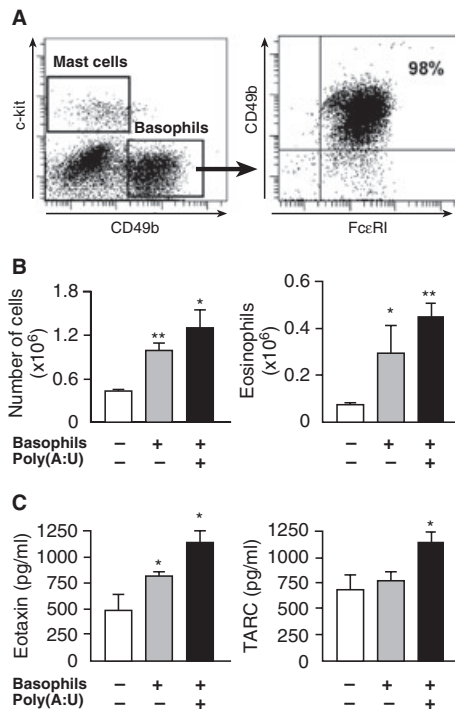


Figure 3 Adoptive transfer of basophils exacerbates allergic inflammation. (A) Preparation of purified bone marrow-derived basophils (BMDb). CD49b⁺c-kit[−] cells were electronically sorted from basophil-enriched populations generated during 7–9 days of culture in the presence of IL-3. (B) After immunization, J α 18^{−/−} mice were divided into three groups: one that received no further treatment and served as control and the other two injected with 10⁶ sorted basophils that had been pretreated with and not with poly(A:U). Total cells and eosinophils in BALF, eotaxin, and TARC levels were quantified in lung (37). $n = 6$. ** $P \leq 0.01$; * $P \leq 0.05$.

OVA-induced basophil recruitment to the liver from 1.9% to 0.1% (Fig. 4A). Whereas hyperreactivity was not significantly modified (data not shown), basophil depletion resulted in a less marked asthma response evidenced by reduced eosinophil recruitment to BALF and lower pulmonary cytokine and chemokine production (Fig. 4B). Poly(A:U)-induced total cell and eosinophil infiltration was abolished after depletion of basophils, proving their critical role in cellular recruitment (Fig. 4C). Taken together, these data support the conclusion that a synthetic analogue of double-stranded viral antigen can target basophils directly to promote their typical pro-T_H2 activities and increase their recruitment *in vivo*, thereby exacerbating experimental allergic asthma.

Discussion

Our study provides the first evidence that the dsRNA poly(A:U) targets basophils to promote their activation in terms of histamine and T_H2 cytokine production and increase their survival. Using a murine model of allergic asthma, we

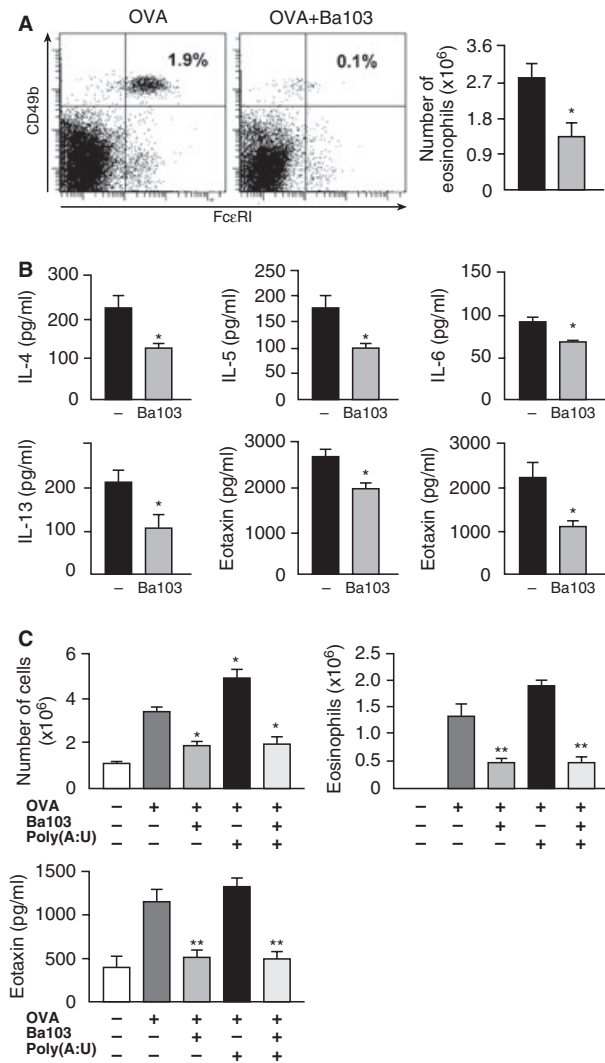


Figure 4 Basophil depletion alleviates allergic inflammation. (A) Reduced eosinophil counts in BALF after basophil depletion. Mice received two daily injections of 5 μ g Ba103 for three days before OVA aerosol challenge. Basophil depletion was verified in the liver by flow cytometry, and eosinophils were counted in BALF. (B) Decreased T_H2 cytokine levels in BALF of basophil-depleted immunized and OVA-challenged mice. Cytokines were measured in BALF 24 h after the last challenge. (C) Abrogation of OVA- or OVA+poly(A:U)-induced increase in eosinophil infiltration after basophil depletion. These experiments were performed twice using 4–6 mice per group. One representative experiment is shown. ** $P \leq 0.01$; * $P \leq 0.05$.

established likewise that this effect is relevant *in vivo* as it enhances the peripheral recruitment and functions of basophils, which in turn contribute directly to the severity of the disease by enhancing asthma syndromes like eosinophil infiltration and T_H2-type cytokine production.

In vivo treatment with poly(A:U) enhanced the incidence of basophils in blood and liver where they conserved their functional characteristics, that is, their capacity to produce

histamine and cytokines in response to IL-3. Even though other mononuclear cells were recruited to the liver upon treatment with poly(A:U), basophils were the major source of IL-4 and IL-6 in response to IL-3. Indeed, the only other potential target of IL-3 in terms of IL-4 and IL-6 production could be mast cells, which are not increased in the liver of mice having received poly(A:U), while the increased responsiveness to IL-3 after treatment with poly(A:U) is consistent with the increased incidence of basophils. In accordance with this conclusion, more than 95% pure sorted basophils derived from bone marrow cells cultured for 7–9 days in the presence of IL-3 produce high amounts of histamine IL-4, IL-6, and IL-13.

Our study provides the first demonstration that basophils infiltrate the lung of mice suffering from allergic asthma and proves by adoptive transfer that they mediate OVA-induced eosinophil recruitment and cytokine/chemokine production. These activities were further upregulated upon exposure to poly(A:U) before transfer, suggesting that they were pathophysiologically relevant. Conversely, *in vivo* basophil depletion by Ba103 antibody-alleviated disease syndromes, thus validating the transfer data. In this context, it is noteworthy that the exacerbated asthma syndromes developed by some mouse strains, such as NOD vs C57BL/6 mice (23), are associated with increased basophil activity in terms of IL-4 and histamine production, while the opposite is true in mice displaying a low T_H2 response (J α 18 KO mice vs WT mice). This observation makes sense if one takes the regulatory functions of basophils during allergic airway inflammation into account.

The exacerbation of asthma symptoms, such as increased eosinophil infiltration in BALF in response to poly(A:U), was associated with increased production of several cytokines known for their pro-inflammatory functions, such as IL-13, a central mediator of allergic asthma (25), and IL-6 (26). The role of IL-4 in asthma exacerbation is particularly well established, owing to its capacity to induce the IgE switch, upregulate the expression of vascular cell adhesion molecule-1 (VCAM-1), enhance eosinophil transmigration across the

endothelium, and increase mucus secretion and differentiation of T_H2 lymphocytes (27). Indeed, increased susceptibility to allergic asthma has been linked to polymorphisms in the IL-4 gene promoter and IL-4R signaling, a finding that has already given rise to clinical trials evaluating the effect of naturally occurring antagonists (28). It is also noteworthy that poly(A:U)-treated mice signal in basophils through a TLR3-independent pathway (A.R., L.V.P., and N.T. personal communication).

Asthma exacerbations have been consistently associated with viral respiratory infections (29, 30). Thus, the role of innate immunity through interferon production in response to viral infection and epithelial airway may initiate chronic asthma (31). In animal models, double-stranded RNA also exacerbated pulmonary allergic reactions (32) through activation of various cell subsets, such as DCs, epithelial cell (33), IL-17A-producing iNKT cells (34), and inflammatory mediators such as histamine, chemokines, and cytokines like IL-6, IL-17, IL-33, etc. (35). In conclusion, we postulate that the asthma exacerbation associated with viral infections might be mediated, at least in part, by basophils through their capacity to skew the antiviral response toward a T_H2 cytokine profile. Our findings provide new insights into the complex mechanisms that regulate the severity of allergic diseases and their paradoxical aggravation by viral infections.

To note: During the reviewing process, a report was published showing that basophil recruitment in lungs aggravated airway inflammation (36).

Acknowledgments

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Conflict of interest

None.

References

- Karasuyama H, Mukai K, Obata K, Tsujimura Y, Wada T. Nonredundant roles of basophils in immunity. *Annu Rev Immunol* 2011;**29**:45–69.
- Casolaro V, Spadaro G, Marone G. Human basophil releasability. VI. Changes in basophil releasability in patients with allergic rhinitis or bronchial asthma. *Am Rev Respir Dis* 1990;**142**:1108–1111.
- Kepley CL, McFeeley PJ, Oliver JM, Lipscomb MF. Immunohistochemical detection of human basophils in postmortem cases of fatal asthma. *Am J Respir Crit Care Med* 2001;**164**:1053–1058.
- Koshino T, Teshima S, Fukushima N, Takaishi T, Hirai K, Miyamoto Y et al. Identification of basophils by immunohistochemistry in the airways of post-mortem cases of fatal asthma. *Clin Exp Allergy* 1993;**23**:919–925.
- Iliopoulos O, Baroody FM, Naclerio RM, Bochner BS, Kagey-Sobotka A, Lichtenstein LM. Histamine-containing cells obtained from the nose hours after antigen challenge have functional and phenotypic characteristics of basophils. *J Immunol* 1992;**148**:2223–2228.
- Noben-Trauth N, Shultz LD, Brombacher F, Urban JF Jr, Gu H, Paul WE. An interleukin 4 (IL-4)-independent pathway for CD4⁺ T cell IL-4 production is revealed in IL-4 receptor-deficient mice. *Proc Natl Acad Sci U S A* 1997;**94**:10838–10843.
- Arinobu Y, Iwasaki H, Akashi K. Origin of basophils and mast cells. *Allergol Int* 2009;**58**:21–28.
- Schneider E, Salachas F, Lemoine FM, Arnould A, Machavoine F, Ploemacher RE et al. Murine hematopoietic progenitor cells produce IL-6 in response to IgE. *Exp Hematol* 1995;**23**:353–361.
- Schneider E, Lemoine FM, Breton-Gorius J, Machavoine F, Arnould A, Cramer EM et al. IL-3-induced coexpression of histidine decarboxylase, IL-4 and IL-6 mRNA by murine basophil precursors. *Exp Hematol* 1999;**27**:1010–1018.
- Siracusa MC, Saenz SA, Hill DA, Kim BS, Headley MB, Doering TA et al. TSLP promotes interleukin-3-independent basophil haematopoiesis and type 2 inflammation. *Nature* 2011;**477**:229–233.

11. Schneider E, Petit-Bertron AF, Bricard R, Levasseur M, Ramadan A, Girard JP et al. IL-33 activates unprimed murine basophils directly in vitro and induces their in vivo expansion indirectly by promoting hematopoietic growth factor production. *J Immunol* 2009;**183**:3591–3597.
12. Paul WE, Zhu J. How are T(H)2-type immune responses initiated and amplified? *Nat Rev Immunol* 2010;**10**:225–235.
13. Falcone FH, Zillikens D, Gibbs BF. The 21st century renaissance of the basophil? Current insights into its role in allergic responses and innate immunity. *Exp Dermatol* 2006;**15**:855–864.
14. Oh K, Shen T, Le Gros G, Min B. Induction of Th2 type immunity in a mouse system reveals a novel immunoregulatory role of basophils. *Blood* 2007;**109**:2921–2927.
15. Sokol CL, Chu NQ, Yu S, Nish SA, Laufer TM, Medzhitov R. Basophils function as antigen-presenting cells for an allergen-induced T helper type 2 response. *Nat Immunol* 2009;**10**:713–720.
16. Perrigoue JG, Saenz SA, Siracusa MC, Allenspach EJ, Taylor BC, Giacomini PR et al. MHC class II-dependent basophil-CD4⁺ T cell interactions promote T(H)2 cytokine-dependent immunity. *Nat Immunol* 2009;**10**:697–705.
17. Yoshimoto T, Yasuda K, Tanaka H, Nakahira M, Imai Y, Fujimori Y et al. Basophils contribute to T(H)2-IgE responses in vivo via IL-4 production and presentation of peptide-MHC class II complexes to CD4⁺ T cells. *Nat Immunol* 2009;**10**:706–712.
18. Jackson DJ, Johnston SL. The role of viruses in acute exacerbations of asthma. *J Allergy Clin Immunol* 2010;**125**:1178–1187.
19. Stensballe LG, Simonsen JB, Thomsen SF, Larsen AM, Lysdal SH, Aaby P et al. The causal direction in the association between respiratory syncytial virus hospitalization and asthma. *J Allergy Clin Immunol* 2009;**123**:131–137 e131.
20. Sugiyama T, Hoshino K, Saito M, Yano T, Sasaki I, Yamazaki C et al. Immunoadjuvant effects of polyadenylic:polyuridylic acids through TLR3 and TLR7. *Int Immunol* 2008;**20**:1–9.
21. Dy M, Lebel B, Schneider E. Presence of histaminase in fetal calf sera. *J Immunol Methods* 1982;**48**:261–265.
22. Schneider E, Machavoine F, Pleau JM, Bertron AF, Thurmond RL, Ohtsu H et al. Organic cation transporter 3 modulates murine basophil functions by controlling intracellular histamine levels. *J Exp Med* 2005;**202**:387–393.
23. Araujo LM, Lefort J, Nahori MA, Diem S, Zhu R, Dy M et al. Exacerbated Th2-mediated airway inflammation and hyperresponsiveness in autoimmune diabetes-prone NOD mice: a critical role for CD1d-dependent NKT cells. *Eur J Immunol* 2004;**34**:327–335.
24. Obata K, Mukai K, Tsujimura Y, Ishiwata K, Kawano Y, Minegishi Y et al. Basophils are essential initiators of a novel type of chronic allergic inflammation. *Blood* 2007;**110**:913–920.
25. Wills-Karp M, Luyimbazi J, Xu X, Schofield B, Neben TY, Karp CL et al. Interleukin-13: central mediator of allergic asthma. *Science* 1998;**282**:2258–2261.
26. Yokoyama A, Kohno N, Fujino S, Hamada H, Inoue Y, Fujioka S et al. Circulating interleukin-6 levels in patients with bronchial asthma. *Am J Respir Crit Care Med* 1995;**151**:1354–1358.
27. Ying S, Meng Q, Barata LT, Robinson DS, Durham SR, Kay AB. Associations between IL-13 and IL-4 (mRNA and protein), vascular cell adhesion molecule-1 expression, and the infiltration of eosinophils, macrophages, and T cells in allergen-induced late-phase cutaneous reactions in atopic subjects. *J Immunol* 1997;**158**:5050–5057.
28. Borish LC, Nelson HS, Corren J, Bensch G, Busse WW, Whitmore JB et al. Efficacy of soluble IL-4 receptor for the treatment of adults with asthma. *J Allergy Clin Immunol* 2001;**107**:963–970.
29. Papadopoulos NG, Christodoulou I, Rohde G, Agache I, Almqvist C, Bruno A et al. Viruses and bacteria in acute asthma exacerbations—a GA(2) LEN-DARE systematic review. *Allergy* 2011;**66**:458–468.
30. Busse WW, Lemanske RF Jr, Gern JE. Role of viral respiratory infections in asthma and asthma exacerbations. *Lancet* 2010;**376**:826–834.
31. Holgate ST. The sentinel role of the airway epithelium in asthma pathogenesis. *Immunol Rev* 2011;**242**:205–219.
32. Stowell NC, Seideman J, Raymond HA, Smalley KA, Lamb RJ, Egenolf DD et al. Long-term activation of TLR3 by poly(I:C) induces inflammation and impairs lung function in mice. *Respir Res* 2009;**10**:43.
33. Torres D, Dieudonne A, Ryffel B, Vilain E, Si-Tahar M, Pichavant M et al. Double-stranded RNA exacerbates pulmonary allergic reaction through TLR3: implication of airway epithelium and dendritic cells. *J Immunol* 2010;**185**:451–459.
34. Vultaggio A, Nencini F, Pratesi S, Petroni G, Romagnani S, Maggi E. Poly(I:C) promotes the production of IL-17A by murine CD1d-driven invariant NKT cells in airway inflammation. *Allergy* 2012;**67**:1223–1232.
35. Yamaya M. Virus infection-induced bronchial asthma exacerbation. *Pulm Med* 2012;**2012**:834826.
36. Wakahara K, Van VQ, Baba N, Begin P, Rubio M, Delespesse G et al. Basophils are recruited to inflamed lungs and exacerbate memory Th2 responses in mice and humans. *Allergy* 2013;**68**:180–189.