Invited review article

Emerging roles of basophils in allergic inflammation

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A R T I C L E   I N F O

Article history:
Received 3 April 2017
Received in revised form 12 April 2017
Accepted 13 April 2017
Available online 11 May 2017

Keywords:
Basophil
IgE-mediated chronic allergic inflammation
IL-4
Protease
Th2 differentiation

Abstract

Basophils have long been neglected in immunological studies because they were regarded as only minor relatives of mast cells. However, recent advances in analytical tools for basophils have clarified the non-redundant roles of basophils in allergic inflammation. Basophils play crucial roles in both IgE-dependent and -independent allergic inflammation, through their migration to the site of inflammation and secretion of various mediators, including cytokines, chemokines, and proteases. Basophils are known to produce large amounts of IL-4 in response to various stimuli. Basophil-derived IL-4 has recently been shown to play versatile roles in allergic inflammation by acting on various cell types, including macrophages, innate lymphoid cells, fibroblasts, and endothelial cells. Basophil-derived serine proteases are also crucial for the aggravation of allergic inflammation. Moreover, recent reports suggest the roles of basophils in modulating adaptive immune responses, particularly in the induction of Th2 differentiation and enhancement of humoral memory responses. In this review, we will discuss recent advances in understanding the roles of basophils in allergic inflammation.

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Introduction

Basophils are the least common granulocytes, representing less than 1% of peripheral blood leukocytes in both mice and humans. Basophils share some features with tissue-resident mast cells,
including basophilic granules in their cytoplasms and expression of the high-affinity IgE receptor FcεRI on their cell surfaces. Therefore, basophils have long and erroneously been considered to serve redundant roles with mast cells. Nevertheless, basophils and mast cells differ in several aspects, including their localization and life span. Basophils circulate in the peripheral blood and migrate to sites of inflammation, whereas mast cells reside in the peripheral tissues and are rarely detected in blood. Moreover, the life span of basophils (~60 h) is much shorter than that of mast cells (~2–3 weeks). Despite these differences that suggest functional differences between basophils and mast cells, the non-redundant roles of basophils have not been identified until recently, partly because of the rarity of basophils and lack of tools for basophil research. The recent development of novel analytical tools, including basophil-depleting antibodies and genetically-engineered mice deficient for basophils, have revealed non-redundant functions of basophils in various immune responses such as chronic allergic inflammation and protective immunity against parasites. Moreover, a recent report showed that expression profiles of basophils are much different from those of mast cells, indicating their distinct roles in immune responses. In this review, we focus on recent advances in our understanding of basophils in allergic inflammation.

Roles of basophils in allergic inflammation

IgE-dependent allergic inflammation

IgE-mediated chronic allergic inflammation (IgE-CAI) in the skin

Infiltration of basophils into inflammatory sites has been reported in various types of allergic inflammation, including atopic dermatitis, allergic rhinitis, and asthma. However, until recently, the contribution of basophils in allergic reactions has remained obscure. Our group has identified non-redundant roles of basophils in a mouse model of IgE-CAI.

In this model, mice were passively sensitized with antigen-specific IgE and then their ears were subcutaneously injected with antigens. Consequently, mice exhibited mast cell-dependent biphasic ear swelling (comprising immediate-phase and late-phase ear swelling), followed by severe ear swelling that peaked on days 3–4 after challenge. This delayed-onset ear swelling accompanied massive infiltration of inflammatory cells, including eosinophils, neutrophils, and macrophages, and hyperplastic epidermis with hyperkeratosis. We designated this delayed-onset response IgE-CAI. IgE-CAI can be elicited even in the absence of mast cells or T cells, suggesting that neither mast cells nor T cells are essential for the development of IgE-CAI. Importantly, adoptive transfer of basophils into FcεRI-deficient mice can reconstitute IgE-CAI, even though basophils account for only ~2% of skin-infiltrating cells, demonstrating the pivotal role of basophils in this reaction. Later studies further confirmed the indispensable role of basophils in IgE-CAI by using basophil-specific depletion antibody (anti-CD200R3) or genetically engineered-mice that specifically lack basophils (Mcpt8Cre mice, diphtheria toxin (DT)-treated Mcpt8DTR mice, and DT-treated Bas-TRECK mice). Notably, depletion of basophils even during the progress of inflammation resulted in the suppression of ear swelling and a drastic reduction in inflammatory cell infiltration, suggesting the effectiveness of basophil-targeted therapy. Therefore, basophils appear to act as initiators of inflammation, recruiting other inflammatory cells such as eosinophils and neutrophils. We postulate the following scenario in IgE-CAI pathogenesis (Fig. 1): First, a small number of basophils are recruited to skin lesions by unknown mechanisms. IgE-bound basophils are activated by antigens, causing the release of various inflammatory mediators, including chemokines, promoting the migration of inflammatory cells, including neutrophils, eosinophils, monocytes and basophils. On the other hand, basophil-derived serine proteases such as mMCP-11 act on serum proteins to generate chemotactic factors, leading to the recruitment of inflammatory cells. Thus basophil-derived inflammatory mediators induce further recruitment of inflammatory cells, leading to chronic allergic inflammation.

Fig. 1. Roles of basophils as initiator of chronic allergic inflammation. In antigen-sensitized mice, circulating basophils are armed with antigen-specific IgE. After administration of antigens into ear skin, a small number of basophils infiltrate into skin lesions and activated by antigens, leading to the release of a variety of mediators. Cytokines and other mediators produced by activated basophils acts on skin-resident cells, including fibroblasts, endothelial cells, and ILCs. Stimulated skin-resident cells secrete substantial amount of chemokines, promoting the migration of inflammatory cells, including neutrophils, eosinophils, monocytes and basophils. On the other hand, basophil-derived serine proteases such as mMCP-11 act on serum proteins to generate chemotactic factors, leading to the recruitment of inflammatory cells. Thus basophil-derived inflammatory mediators induce further recruitment of inflammatory cells, leading to chronic allergic inflammation.
cytokines, chemokines, and proteases. Basophil-derived mediators may act on skin-resident cells (fibroblasts, endothelial cells, and others), leading to the recruitment of large number of inflammatory cells. Basophil-derived proteases generate chemotactic factors by acting on serum proteins, which can induce further basophil infiltration, as described in a later section. Thus, the infiltration of small numbers of basophils leads to the aggravation of inflammation. Basophils are required to migrate from blood to tissue, and therefore basophil-dependent inflammation may show a delayed onset, in contrast to acute-onset mast cell-dependent inflammation.

Basophils have been shown to play essential roles in IgE-dependent skin allergy models other than IgE-CAI.15,16 Likewise, in the course of repeated parasitic infections, IgE-armored basophils infiltrate into sites of infection and play key roles in generating protective immunity against parasites in an IgE-dependent manner.17

IgE-dependent airway inflammation – rhinitis and asthma

In addition to skin inflammation, basophils contribute to several models of IgE-dependent airway inflammation. Recent reports have shown that depletion of basophils improves asthma symptoms in models of allergic rhinitis.18,19 In one model, mast cells are also crucial for the development of allergic rhinitis.20,21 and it is assumed that sequential engagement of mast cells and basophils is important to elicit nasal immune responses.20 Allergen exposure stimulates mast cells in nasal mucosa to release histamine, which acts on the histamine H4 receptor on basophils, leading to migration of basophils into nasal tissues. Activated basophils in nasal tissues release various mediators to induce both early- and late-phase nasal reactions. In contrast to the allergic rhinitis model, the role of basophils remains ill-defined in the IgE-dependent allergic asthma model. One report indicated that mast cells, but not basophils, are essential for the development of airway inflammation in allergic asthma,22 whereas other reports have shown the importance of basophils in airway inflammation and Th2 responses.23,24 These reports suggest that the relative importance of basophils and mast cells in allergic asthma differs depending on experimental conditions. Because basophils play crucial roles in delayed-onset responses in IgE-CAI, it can be postulated that basophils may also play important roles in asthma models using chronic allergen challenge.

Human allergic disorders

Whether basophils play crucial roles in human allergic disorders remains unclear. However, recent clinical experiences with anti-IgE antibody therapies (e.g., Omalizumab) provide some evidence of their role in human diseases. Omalizumab binds to the FcεRI on basophils and mast cells. Downregulation of FcεRI expression on basophils occurs within 2 weeks, whereas that on mast cells typically occurs after 8 weeks.25,26 Therefore, it is expected that the therapeutic effects of omalizumab in basophil-mediated allergic reactions can be observed as early as 1–2 weeks.

Chronic idiopathic urticaria (CIU) is defined as itchy hives that last for at least 6 weeks and that have no apparent external trigger. Although H1-antihistamines are widely prescribed for the initial treatment of patients with CIU, the majority of patients do not respond to these drugs, even at the maximum licensed dose.27 Recently, the administration of omalizumab has been shown to diminish the clinical symptoms of CIU in phase 3 clinical trials, and omalizumab is approved by the US Food and Drug Administration (FDA) and European Medicines Agency (EMA) as a novel drug for patients with CIU.28,29 Of note, the therapeutic effects of omalizumab on patients with CIU can be observed within 1 week, suggesting the involvement of basophils in the pathogenesis of CIU.28 Several observations support the role of basophils in CIU.30 Patients with CIU present blood basopenia, which is believed to be the result of basophil migration into skin lesions. Consistent with this, a recent report has shown that basopenia is significantly ameliorated with omalizumab treatment.31 Furthermore, basophils in patients with CIU show paradoxical suppression of FcεRI-mediated histamine release, which reverses with disease remission.32 A recent report showed that sera from patients with CIU suppressed the activation of basophils from healthy donors, suggesting that unknown suppressive factors are present in the sera of patients with CIU.33 Taken together, results of these studies suggest that IgE and basophils play essential roles in the pathogenesis of chronic urticaria.

In contrast to CIU, contribution of basophils in other IgE-mediated human allergic disorders including allergic rhinitis and asthma remain poorly understood, although some evidences suggest the role of basophils in these diseases.34,35

IgE-independent allergic inflammation

Basophils are also crucial in IgE-independent allergic inflammation in mouse models, including asthma induced by allergenic proteases,36,37 irritant contact dermatitis,38 atopic dermatitis (AD)-like skin inflammation,39–41 and eosinophilic esophagitis (EoE).42,43 (Fig. 2). Basophils respond to a variety of innate immune-related stimuli other than IgE with antigen stimulation.44 IL-3, a key cytokine for basophil survival, induces IL-4 production in basophils through interaction with the Fc receptor common γ-chain (FcγRγ), which is constitutively associated with the IL-3 receptor.45–47 IL-18, Toll-like receptor (TLR) ligands, including lipopolysaccharide (LPS) and peptidoglycans (PGNs), induce IL-4 production by basophils in the presence of IL-3.48 Several allergenic proteases such as papain and house dust mite-derived proteases induce activation of basophils.49,50 A recent report showed that extracellular ATP can also modulate basophil activation.51

Recent findings indicate that basophils have two distinct subpopulations depending on cytokine milieu, namely thymic stromal lymphopoietin (TSLP)-elicited basophils and IL-3-elicited basophils.52 TSLP-elicited basophils show higher expression of receptors for IL-3, IL-18, and IL-33, members of the IL-1 family of cytokines, also induce IL-4 production in basophils.53–55 Toll-like receptor (TLR) ligands, including lipopolysaccharide (LPS) and peptidoglycans (PGNs), induce IL-4 production by basophils in the presence of IL-3.56–58 Several allergenic proteases such as papain and house dust mite-derived proteases induce activation of basophils.59–61 A recent report showed that extracellular ATP can also modulate basophil activation.51

TSLP is reported to be highly expressed in keratinocytes from patients with AD, and polymorphisms in TSLP are directly associated with AD.51,52 TSLP upregulation induced by a transgene specifically expressed in skin or repeated application of a chemical can trigger AD-like skin inflammation in mice.53–55 In some TSLP-dependent AD models, depletion of basophils results in the amelioration of skin inflammation, suggesting the contribution of TSLP-elicited basophils in these models.56–58

Gain-of-function polymorphisms in TSLP are also associated with EoE, a food allergy-associated inflammatory disease strongly associated with AD.59–61 Mouse models of EoE are established by sensitization with food allergens in barrier-disrupted skin lesions, followed by oral allergen challenge.62,63 Development of EoE in these models is dependent on basophils and TSLP, and basophil infiltration is detected in esophageal lesions of mice and humans.62,63 Recent study demonstrated the significance of the IL-33-basophil axis in this model.63 Depletion of basophils abolished the
Basophil-dependent allergic inflammation

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**Examples of Mouse Models**
- IgE-mediated chronic allergic inflammation (IgE-CAI) (Refs. 10-12)
- IgE-dependent allergic rhinitis (Refs. 19-20)
- Protease-induced asthma (Refs. 36-37)
- Irritant contact dermatitis (Ref. 38)
- AD-like skin inflammation (Refs. 39-41)
- EoE-like inflammation (Refs. 42-43)

**Related Human Disease**
- Chronic idiopathic urticaria (CIU)
- Eosinophilic esophagitis (EoE)

![Fig. 2. Involvement of basophils in IgE-dependent and IgE-independent allergic inflammation.](image)

Development of EoE, and EoE was recovered by adoptive transfer of basophils from WT but not IL-33 receptor-deficient mice. Moreover, IL-33 receptor expression is increased in esophagi of patients with EoE. Based on these results, it can be postulated that TSLP-elicited basophils infiltrate the esophagus and that basophils activated by IL-33 and other stimuli initiate eosinophilic inflammation.

The significance of basophils and TSLP was also demonstrated in food allergy models induced by epicutaneous sensitization with allergens. TSLP and basophils play key roles in allergen-specific IgE production during the sensitization phase, while mast cells are important for the development of food allergy after challenge. Of note, the significance of TSLP receptors on dendritic cells (DCs) has been demonstrated in one model, which suggests that basophils and DCs work together to induce Th2 cells and IgE production, as further discussed in later sections.

**Basophil-derived effector molecules inducing allergic inflammation**

Although it is now widely appreciated that basophils contribute to several mouse allergy models as described above, it has remained unclear how basophils induce inflammation after activation. Recently, several studies shed some light on this issue. In this section, we will focus on the effects of basophil-derived cytokines, proteases, and other mediators on allergic inflammation.

**Cytokines and chemokines**

**Role of basophil-derived IL-4 in allergic inflammation**

Both mouse and human basophils are known to produce large quantities of IL-4 in response to various stimuli. Roles of basophil-derived IL-4 in allergic inflammation have been extensively studied in recent years. Several lines of evidence indicate that basophil-derived IL-4 acts on a variety of cell types to induce eosinophil infiltration (Fig. 3). Indeed, basophil depletion leads to reduced eosinophil recruitment to the site of inflammation in several allergy models. In a co-culture system, basophil-derived IL-4 and TNF-α stimulate fibroblasts to secrete eotaxin-1 (CCL11) and RANTES (CCL5), causing the migration of eosinophils. In an IgE-dependent skin inflammation model, basophil-derived IL-4 induces the expression of adhesion molecules such as vascular cell adhesion molecule-1 (VCAM-1) in endothelial cells, which facilitates transmigration of eosinophils into inflammatory sites. Indeed, basophil-specific IL-4-deficient mice showed impaired induction of VCAM-1 expression on endothelial cells and reduced infiltration of eosinophils in skin lesion. In a papain-induced airway inflammation model, basophil-derived IL-4 stimulates lung-resident group 2 innate lymphoid cells (ILC2s), leading to the secretion of larger amounts of IL-5, IL-13, and CCL11, thus inducing the migration of eosinophils. Mice that specifically lack basophil-derived IL-4 showed reduced secretion of cytokines in ILC2s and impaired recruitment of eosinophils in lungs. Another group has reported similar findings in an allergic skin inflammation model, in which basophil-derived IL-4 induces the activation of skin-resident ILC2s, thus promoting eosinophilic migration into skin lesions.

**Role of other cytokines/chemokines derived from basophils**

Besides IL-4, basophils from mice and humans produce various cytokines and chemokines such as IL-6, IL-13, TNF-α, MIP-1α (CCL3), and MIP-1β (CCL4). Basophil-derived IL-6 was recently reported to be important for Th17 cell differentiation and humoral memory responses, but further investigation is required to define the significance of basophil-derived IL-6 in these responses. Roles of other cytokines/chemokines in allergic reactions remain largely unclear. A recent report suggested the contribution of basophil-derived chemokines to tumor rejection. During the rejection of melanoma tumors, depletion of regulatory T cells (Treg) induces infiltration of basophils into tumors, which promotes CD8+ T cell recruitment by basophil-derived CCL3 and CCL4, resulting in efficient rejection of tumors. In addition to the cytokines mentioned above, basophils can also produce amphiregulin, an epidermal growth factor (EGF)-like cytokine, upon stimulation with IL-3 produced by T cells. Basophil-derived amphiregulin is a key participant in UVB irradiation-induced immune suppression by enhancing the suppressive function of Treg cells.

**Effects of basophil-derived proteases on allergic inflammation**

Both basophils and mast cells store serine proteases in their granules and release them in response to various stimuli.
Importantly, recent studies have revealed that basophils and mast cells in mice possess different serine proteases. We and other groups have found that basophils rather than mast cells preferentially express mouse mast cell protease 11 (mMCP-11) and mMCP-8, while mMCP-6 and mMCP-7 are selectively expressed by mast cells. The roles of mast cell-selective mMCP-6 and mMCP-7 have been extensively studied, and these proteases play significant roles in protection against bacterial infections and the development of inflammatory diseases. In contrast, the physiological significance of basophil-selective mMCP-8 and mMCP-11 has remained elusive until recently.

We found basophil-derived tryptase mMCP-11 plays crucial roles in IgE-CAI responses (Fig. 4). mMCP-11-deficient mice showed an ameliorated IgE-CAI response, with reduction in ear swelling, microvascular permeability, and infiltration of leukocytes, including eosinophils, neutrophils, and macrophages. In addition, injection of recombinant mMCP-11 protein into mouse ears induced cutaneous ear swelling with increased microvascular permeability, and three consecutive injections of recombinant protein induced cellular infiltration. Of note, mMCP-11 also induces leukocyte recruitment in a transwell migration assay in vitro. Based on observations that this leukocyte migration requires serum in culture media and that this migration activity in response to mMCP-11 is dependent on protease activity, mMCP-11 appears to cleave serum proteins, and proteolytic products induce the migration of leukocytes, including basophils. Inhibitor experiment further revealed that mMCP-11-elicted leukocyte migration is dependent on G protein-coupled receptors (GPCRs). These findings indicate that mMCP-11, which is released from basophils upon degranulation, promotes further migration of basophils, leading to further release of mMCP-11 in affected tissues.

Our recent report determined the function of mMCP-8 using recombinant proteins (Fig. 4). Similar to mMCP-11, administration of mMCP-8 into mouse ears induced ear swelling with increased microvascular permeability and leukocyte infiltration in a protease activity-dependent manner. However, unlike mMCP-11, mMCP-8 did not possess chemotactic activity in vitro, but rather induced chemokine expression in skin-resident cells to induce leukocyte infiltration. Thus, basophil-derived serine protease mMCP-8 and mMCP-11 cooperatively induce allergic inflammation by promoting increased microvascular permeability and leukocyte infiltration through different mechanisms.

**Effects of other mediators — histamine, PAF and lipid mediators**

Histamine is the major chemical mediator stored in granules of basophils. Histamine release from basophils is widely measured in clinical tests for food allergies. However, the significance of histamine released from basophils remains ill defined. Indeed, mast cells and histamines play crucial roles in IgE-induced systemic anaphylaxis, whereas basophils are dispensable for this reaction. In contrast, basophils play crucial roles in IgG-induced anaphylaxis by releasing platelet-activating factor (PAF). However, following studies also demonstrated the significance of neutrophils and macrophages in IgG-induced anaphylaxis under different experimental conditions, indicating that the relative contribution of basophils, neutrophils, and macrophages in IgG-dependent anaphylaxis can differ depending on experimental conditions. A recent study indicates that the IgG subclass and its binding capacity to Fcγ receptors determine the contribution of basophils, neutrophils, and macrophages to IgG-induced anaphylaxis.

Eicosanoids, lipid mediators generated from arachidonic acids, are also released from basophils upon stimulation. Basophils have
Basophils long been considered to release a much narrower range of eicosanoids, compared to mast cells. However, our recent study revealed that basophils can produce 5-lipoxygenase (5-LOX) metabolites and cyclooxygenase (COX) metabolites upon stimulation. Furthermore, the production of COX metabolites from mast cells and basophils are differentially regulated by COX-1 and COX-2, leading to the distinct time course of metabolite production, namely early-phase after stimulation in mast cells while late-phase after stimulation in basophils.

Regulation of other immune cells by basophils

Recent studies have revealed that basophils contribute to immune regulation through their interaction with other immune cells, including T cells, B cells, monocytes and macrophages. Basophils play a role in the regulation of acquired immunity, especially in the induction of Th2 differentiation and amplification of humoral memory responses. In addition, basophil-derived IL-4 is involved in the induction of M2-type macrophages, leading to the resolution of inflammation and protection immunity against parasites. In this section, we will focus on the immune modulatory functions of basophils.

Regulation of T cells by basophils

Th2 cells are crucial for protective immunity against extracellular parasites and in several allergic reactions. IL-4 is widely recognized to play critical roles in the differentiation of naïve T cells into Th2 cells. The cellular source of IL-4 responsible for initial Th2 differentiation has long been a matter of debate, because several cell types, including natural killer T cells, T cells, mast cells, eosinophils, and basophils, are known to be able to produce IL-4. Basophils rapidly release large amounts of IL-4 in response to a variety of stimuli, as described in previous sections. Additionally, analyses using IL-4-green fluorescence protein (GFP) reporter mice revealed that basophils are the major source of IL-4 during helminth infections. Indeed, co-culture of naïve T cells with wild-type (WT) basophils in the presence of DCs induces robust production of IL-4 in T cells, while co-culture with IL-4-deficient basophils fails to do so. These results highlight the importance of basophils as a provider of IL-4 for Th2 differentiation in vitro. The role of basophils in Th2 differentiation through the provision of IL-4 was further supported in a Th2 differentiation model caused by allergic proteases such as papain. Basophils were transiently recruited to draining lymph nodes 1 day before the peak of Th2 differentiation in response to subcutaneous injection of papain. Basophils were localized in the T cell zone of draining lymph nodes and expressed IL-4 in response to papain stimulation. Depletion of basophils abolished Th2 differentiation induced by papain immunization. Therefore, it was assumed that DCs act as antigen-presenting cells (APCs) to induce Th2 differentiation whereas basophils function as accessory cells in providing IL-4 to T cells.

Following reports from three independent groups demonstrated, under different experimental conditions, that basophils, rather than DCs, function as the critical APCs in driving Th2 differentiation in distinct experimental conditions. In all settings, basophils expressed both major histocompatibility complex class II (MHC-II) and co-stimulatory molecules (CD80, CD86, or CD40) necessary for APC function and could process and present antigens to naïve T cells, leading to Th2 cell differentiation. Depletion of basophils using anti-FcεRI antibody abolished Th2 differentiation in vivo, whereas depletion of DCs using CD11c-DTR chimeric mics did not affect Th2 differentiation in these models.
Furthermore, mice in which MHC-II expression was restricted to DCs did not show Th2 differentiation in vivo. Conversely, adoptive transfer of antigen-pulsed basophils was found to be sufficient for the initiation of Th2 responses. This paradigm shift was greeted with great enthusiasm, but also with criticism. One concern has been the method used for depletion of DCs and basophils. A report suggested the possibility that radioresistant DCs may have remained intact in the DC-depleted chimeric mice. Another report argued that the basophil-depleting anti-FcεRI antibody may also have ablated FcεRI-expressing inflammatory DCs. Indeed, the crucial role of DCs in Th2 differentiation was demonstrated in studies that followed, suggesting that the relative contributions of basophils and DCs to Th2 differentiation vary depending on experimental conditions. Additionally, basophils show relatively low levels of cathepsin S expression, raising concerns about basophils’ ability of process and present antigen. Thus, the significance of MHC-II expression on basophils and the antigen presentation capacity of basophils remain controversial.

One recent report showed that basophils efficiently induce Th2 differentiation in response to peptide antigen rather than protein antigen. Basophils contribute to ovalbumin (OVA) peptide-induced Th2 differentiation, but not OVA protein-induced Th2 differentiation. Basophils have low capacity to take up and process antigens, and thus are unable to present antigens to T cells when protein antigens are administered. In contrast, basophils can induce T cell proliferation and Th2 differentiation when basophils and naive T cells are co-cultured in the presence of peptide antigens. From these results, authors have concluded that properties of antigens, such as proteins, peptides, and hapten, may determine the relative contribution of basophils to Th2 differentiation.

We hypothesized that the difference in the level of MHC-II expression on basophils might determine the contribution of basophils to Th2 differentiation, and investigated MHC-II expression on basophils in various experimental conditions. We clearly detected basophil MHC-II expression under certain conditions. Unexpectedly, MHC-II expression was detected only at the protein level, but not at the transcription level, suggesting that basophils acquire MHC-II molecules from MHC-II-expressing cells such as DCs. Consistently, co-culture of basophils with DCs results in the detection of DC-derived peptide-MHC-II complexes on basophils. Transfer of peptide-MHC-II complexes from DCs to basophils was accompanied by the transfer of plasma membrane patches, suggesting the involvement of trogocytosis in this phenomenon. Trogocytosis of MHC-II from DCs to basophils appears to occur in vivo, because MHC-II expression on basophils isolated from draining lymph nodes was abolished in DC-specific MHC-II-deficient mice during atopic dermatitis-like inflammation. Acquired peptide-MHC-II complexes enable basophils to stimulate antigen-specific T cells and induce IL-4 production in T cells. Thus, basophils can function as Th2-driving APCs through trogocytosis-mediated MHC-II acquisition from DCs (Fig. 5C). The relative contribution of basophils to Th2 cell differentiation may depend on the extent of MHC-II trogocytosis from DCs. This appears to reconcile some discrepancies observed previous studies.

### Regulation of B cells by basophils

Besides their roles in T cell differentiation, basophils have been shown to contribute to the modulation of B cell memory responses in mice and humans. Basophils produce large amounts of IL-4 in secondary immune responses after immunization. However, the role of basophil-derived IL-4 in memory responses, especially antibody production by B cells, has remained elusive until recently. Denzel et al. showed that basophils play crucial roles in humoral immune responses by producing IL-4 and IL-6, which induce a B cell helper phenotype in CD4+ T cells and enhance B cell proliferation and antibody production. Basophils can efficiently capture antigens after primary immunization via antigen-specific IgE and high-affinity IgE receptors on basophils. Basophils are the main source of IL-4 and IL-6 after challenge, as shown in previous studies. Of note, basophil depletion abrogated the production of antigen-specific IgG1 and IgG2a antibody after secondary antigen challenge. The number of antigen-specific B cells also decreased after depletion of basophils. Indeed, in vitro co-culture of activated WT basophils, but not IL-6-deficient basophils, with activated CD4 T cells and B cells enhanced the proliferation of and antibody...
production by B cells, suggesting the importance of basophil-derived IL-6 in B cell help. Additionally, activated basophils induce a B cell helper phenotype in CD4 T cells through IL-4, IL-6, and CD40L expression. Furthermore, Denzel et al. demonstrated the significance of basophils in a vaccination model using pneumococcal surface protein A (PsPA), a component of Streptococcus pneumoniae. Basophil depletion decreased PsPA-specific antibody production and increased the occurrence of sepsis caused by intratracheal infection with S. pneumoniae. Later studies revealed that basophils in mice and humans support plasma cell survival in the bone marrow and spleen and that this is partly dependent on IL-4 and IL-6 produced by basophils. In long-term depletion of basophils after immunization reduced the number of plasma cells in spleens.

Another group discovered a B cell-stimulating function of basophils in response to IgD. Circulating basophils in humans constitutively exhibit abundant surface IgD via unknown receptors on their cell surface. Crosslinking of IgD using anti-IgD monoclonal antibody induces calcium influx and the production of IL-4, IL-13, soluble B cell-activating factor (BAFF), and membrane-bound proliferation-inducing ligand (APRIL), which are known to be B cell-activating factors. Indeed, co-culture of IgD-activated basophils with plasma cells in spleens.101

Recent reports indicate that M2 macrophage generation induced by basophil-derived IL-4 plays key roles in a wide variety of immune responses. We showed that M2 macrophages generated in the skin are important for protection from the helminth Nipponstrongylus brasiliensis (Nb). During secondary Nb infection, IgE-armored basophils infiltrate skin infection sites and trap Nb larvae in skin. Basophil-derived IL-4 promotes the generation of M2 macrophages, which leads to the retention and killing of larvae by arginase-1.102 Additionally, a recent report showed that M2 macrophage generation in response to basophil-derived IL-4 contributes to liver homeostasis after infection with Listeria monocytogenes (Lm). Lm infection induces early cell death of liver-resident Kupffer cells, resulting in anti-bacterial type 1 responses. Kupffer cell death also induces secretion of IL-33 from hepatocytes, leading to IL-4 production by basophils and M2 macrophage generation from monocytes. Differentiated M2 macrophages replace dead Kupffer cells to maintain tissue homeostasis.

Concluding remarks

Basophils have long been neglected in the immunology field because of their similarities with mast cells and their rarity. However, recent advances in the development of analytical tools have accelerated functional research on basophils. Basophils are now accepted to play key roles in various types of IgE-mediated allergic inflammation. Moreover, basophils have also been shown to play crucial roles in TSLP-mediated and IgE-independent allergic inflammation. Basophils have the potential to initiate and expand inflammation through the production of specific cytokines and proteases. Basophils can also induce Th2 differentiation in cooperation with DCs. In addition, basophils play significant roles in the resolution of inflammation by inducing M2 macrophages.

Recent clinical experiences suggest the role of basophils in the pathogenesis of several allergic disorders. Therefore, further elucidation of the molecular mechanisms underlying basophil-mediated allergic diseases will be the good help for the development of novel therapeutic target for allergic diseases.

Acknowledgments

This work is supported by research grants from the Japanese Ministry of Education, Culture, Sports, Science and Technology (Grant number: 15H05786).

Conflict of interest

The authors have no conflict of interest to declare.

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