

Nonredundant Roles for Basophils in Immediate- and Delayed-onset Allergic Reactions

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Abstract: Basophils circulate in the peripheral blood under physiological conditions, and they are recruited to affected tissues in allergic reactions, albeit in small numbers. Because of their rarity (less than 1% of peripheral blood leukocytes are basophils), and their similarity to mast cells, basophils have often been considered the lesser relatives of mast cells. Moreover, because basophils have been so difficult to identify, mice were erroneously believed for a long time to lack them. Therefore, the assumption that basophils have only redundant roles has remained unquestioned until recently. The flow-cytometric identification of basophils in mice and the development of *in vivo* models and reagents useful for their functional analyses have greatly advanced the field of basophil research. Previously unrecognized roles of basophils, distinct from those of mast cells, have been shown in allergic responses and the regulation of acquired immunity. In this review, we mainly focus on roles of basophils in immediate- and delayed-onset allergic reactions. Basophils are crucial initiators, rather than effectors, in the development of IgE-mediated, chronic cutaneous allergic inflammation, which is characterized by the massive infiltration of eosinophils and neutrophils and can be elicited even in the absence of mast cells and T cells. Basophils are dispensable for the induction of IgE-mediated systemic anaphylaxis, unlike mast cells, but play a major role in IgG-mediated passive and active systemic anaphylaxis, through the release of platelet-activating factor in response to stimulation with antigen-IgG immune complexes. Thus, basophils and their products appear to be promising therapeutic targets for allergic disorders.

Key Words: Basophils, anaphylaxis, allergic inflammation.

INTRODUCTION

Basophils are the least common granulocytes, accounting for less than 1% of peripheral blood leukocytes. Even though their lineage, with regard to other hematopoietic cells, and how they differentiate from hematopoietic stem cells in the bone marrow remain uncertain, basophils share several features with mast cells, including the presence of basophilic granules in the cytoplasm, the surface expression of the high-affinity receptor FcεRI, and the release of allergy-inducing chemical mediators such as histamine and leukotrienes [1-3]. Furthermore, because the identification of mouse basophils by conventional methods, such as Giemsa staining, is extremely difficult [4, 5], for a long time people erroneously concluded that mice lack basophils [6]. Therefore, basophils have been the ‘ugly stepchild’ of leukocytes, and most of the attention has focused on mast cells. The discovery in the early 1990s that basophils are an important source of Th2 cytokines, including IL-4 [7-10], greatly changed the view that they are a minor and redundant, circulating variant of tissue-resident mast cells, and suggested that basophils might contribute to allergic responses and to protective immunity against parasites [11-13].

Basophils are often recruited to the site of allergic inflammation, albeit in small numbers [14-19]. However, the direct assessment of their roles in allergic responses was hampered by the absence of suitable models, including mice deficient only in basophils. Recent studies have overcome this obstacle by creating new tools such as basophil-depleting antibodies [20-23], and previously unrecognized roles for basophils have been demonstrated *in vivo* [24-33]. In this review, we highlight the nonredundant roles played by basophils in immediate- and delayed-onset allergic reactions.

I. THE ROLE OF BASOPHILS IN DELAYED-ONSET AND CHRONIC ALLERGIC INFLAMMATION

Basophils in Jones-Mote hypersensitivity and cutaneous basophil hypersensitivity

During the first golden age of basophil research, the 1970s, extensive studies were performed on the cutaneous delayed-type hypersensitivity reaction that is characterized by a massive dermal infiltration of basophils [34]. This reaction is distinct from the classical, delayed-type hypersensitivity (DTH) reaction, and therefore was originally termed “Jones-Mote hypersensitivity” (JMH) in humans [35]. Later, the reaction was called “cutaneous basophil hypersensitivity” (CBH) [36, 37], mainly in guinea pig studies. While the immunization of animals with protein antigens in complete Freund’s adjuvant containing mycobacterial components is usually required to elicit DTH, CBH can be elicited by im-

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munizing animals, particularly guinea pigs, with proteins alone or in incomplete Freund's adjuvant without mycobacterial components, and is induced only soon after the immunization [36]. In DTH, an intradermal challenge with antigens induces a skin reaction characterized by erythema and induration, which reaches its maximal intensity within 24 to 30 hr. The skin remains indurated for 48 to 72 hr. In contrast, CBH is characterized by erythema and slight thickening without apparent induration, peaks 18~24 hr after the antigen challenge, and fades by 48 hr. In typical CBH lesions, basophils comprise 50~90% of the papillary dermal infiltrates [37]. This is in sharp contrast to the DTH lesions, in which basophil recruitment is rarely detected.

CBH reactions exhibit heterogeneous pathogenicity. It was originally reported that the adoptive transfer of lymphocytes, most likely T cells, but not serum from sensitized guinea pigs causes CBH in naïve animals [38], in a manner analogous to DTH, but distinct from the antibody-mediated, immediate hypersensitivity reaction. However, later studies showed that CBH can be induced by the passive transfer of IgG1 or IgE from sensitized guinea pigs to naïve ones [39, 40]. Thus, the mechanism by which the CBH response, including basophil recruitment, is triggered remains uncertain, as does the role of basophils in CBH. The treatment of guinea pigs with anti-basophil serum before the antigen challenge results in increased rather than decreased erythema, concomitant with a reduced number of basophils in the skin lesions [41], suggesting that basophils may play an anti-inflammatory role in CBH.

The golden age of basophil research on JMH and CBH was over by the early 1980s, as judged from the number of publications on these reactions. This may have come, in part, from the above-mentioned unexpected result, which disappointingly argued against a role for basophils as initiators or effectors in CBH. Moreover, detailed analyses of the cellular and molecular mechanisms underlying CBH were hampered by the absence of mouse models of CBH. The typical CBH reaction, i.e., characterized by a massive infiltration of basophils, was reportedly not elicited in mice. This might have been due to the difficulty in identifying mouse basophils in tissue sections [4-6], compared to guinea pig basophils. Therefore, the nature of CBH in mice and the role of basophils in it require reevaluation.

Basophils in antigen-specific, IgE-mediated chronic allergic inflammation in mice

In sensitized individuals, allergen challenge typically induces two sequential allergic responses. The immediate-phase reaction develops within minutes of allergen exposure, while the late-phase reaction occurs within hours. When antibodies of the IgE class are produced against a given allergen in sensitized individuals, they circulate in the peripheral blood and bind to FcεRI on circulating basophils and tissue-resident mast cells. Re-exposure to the same allergen triggers the activation of mast cells and basophils through the cross-linking of IgE-bound FcεRI by the allergen [42]. The major features of the immediate-phase reaction and at least some features of the late-phase reaction are explained by the actions of chemical mediators, cytokines and chemokines, that are released from activated mast cells, and perhaps from basophils [43-45]. By contrast, the roles of IgE, mast cells, and

basophils are less clear in the pathogenesis of chronic allergic inflammation, which can persist for days to years, as observed in chronic allergic disorders, including atopic dermatitis and asthma.

To understand the *in vivo* roles of IgE under physiological and pathological conditions, and the cellular and molecular basis of IgE-mediated allergic reactions, we have created IgE transgenic mice that constitutively produce monoclonal IgE specific to hapten 2,4,6-trinitrophenol (TNP) [46]. Giving these mice an intravenous injection of the corresponding antigen, TNP-conjugated ovalbumin (TNP-OVA), induces antigen-specific systemic anaphylaxis and a drop in body temperature [46], indicating that the transgenic IgE functions *in vivo*. To examine local allergic reactions, we challenged the IgE transgenic mice with an intradermal injection of TNP-OVA or control OVA. Unexpectedly, the TNP-OVA administration induced three waves of ear swelling [24, 47]. The first two were typical immediate-type skin reactions: the early-phase ear swelling occurred within 1 hr of the antigen challenge, followed by a late-phase, milder ear swelling 6-10 hr later. Notably, after the late-phase ear swelling subsided, ear swelling started again on day 1~2 and peaked on day 3~4 post-challenge. This delayed-onset, third ear swelling was more intense than the first and second ones. The ear skin was congested, and the ear swelled to twice its basal thickness or the thickness of ears challenged with control OVA. The time course of ear swelling in the third reaction resembled that observed in the classical DTH reaction mediated by T cells. However, histopathological analysis revealed that the skin lesions in the third reaction were distinct from those observed in DTH, showing a massive infiltration of cells, predominantly eosinophils and neutrophils [24, 47]. Therefore, we designated this antigen-specific, delayed-onset cutaneous reaction, "IgE-mediated chronic allergic inflammation" (IgE-CAI) [24]. We found that a comparable triphasic ear swelling could be elicited even in non-transgenic mice that had been passively sensitized with intravenous injection of anti-TNP IgE one day before the challenge with TNP-OVA [24]. This finding demonstrated that the development of IgE-CAI was not an artifact derived from the transgenic expression of IgE, and made it possible to investigate IgE-CAI in various mutant mice without crossing them to the IgE transgenic mice.

Anti-histamine treatment efficiently suppressed both the immediate- and late-phase ear swelling, but showed no significant effect on the IgE-CAI, which instead responded to immunosuppressants like cyclosporine A or corticosteroids [47], suggesting that T cells might be involved in IgE-CAI development. However, IgE-CAI, including the eosinophilic infiltration of the skin lesions, was elicited normally in T cell-deficient mice [24], which showed that T cells are dispensable for the development of IgE-CAI. Mast cell-deficient mice also exhibited the IgE-CAI response, although they failed to show the early- and late-phase ear swelling, which indicated that mast cells are not essential for IgE-CAI, and that the immediate- and late-phase reactions mediated by mast cells are not prerequisite for the development of IgE-CAI [24].

Two types of IgE receptors are known, the high-affinity receptor FcεRI and the low-affinity receptor CD23 (also

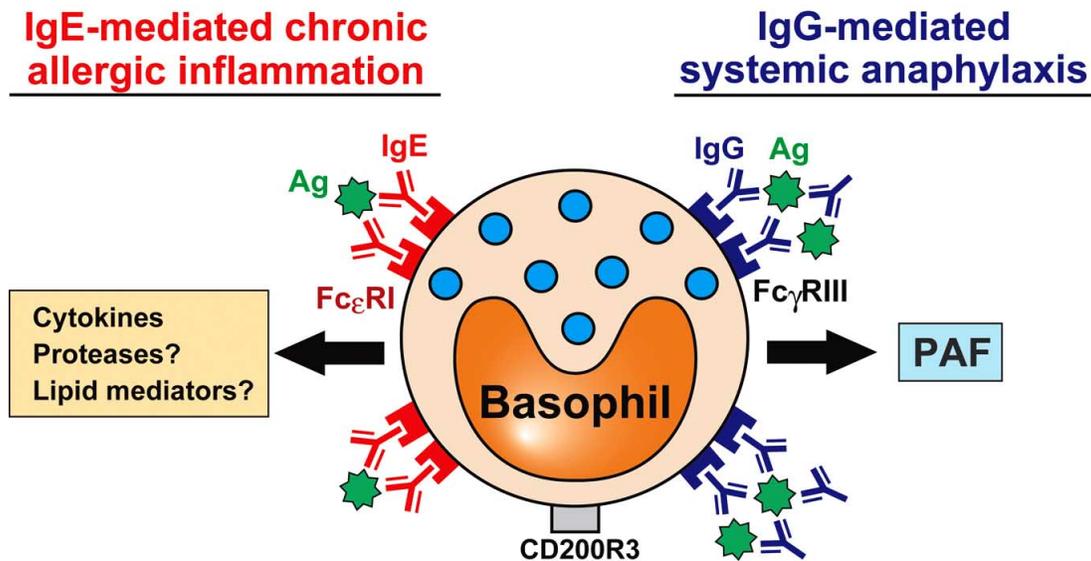


Fig. (1). Crucial roles of basophils in IgE-mediated chronic allergic inflammation and IgG-mediated systemic anaphylaxis.

IgE-mediated chronic allergic inflammation (Left): basophils bearing antigen-specific IgE are recruited into the peripheral tissue. Upon encounter with the relevant antigens, they are activated to secrete soluble factors, including cytokines, proteases, and lipid mediators. These factors act on surrounding tissue-resident cells to induce the chemokine production, which in turn recruits large numbers of proinflammatory cells such as eosinophils and neutrophils, leading to chronic allergic inflammation. IgG-mediated systemic anaphylaxis (right): circulating basophils efficiently capture antigen-IgG immune complexes through Fc γ RIII, and are activated to release PAF, which elicits systemic anaphylaxis.

known as Fc ϵ RII). CD23^{-/-} mice developed IgE-CAI, whereas Fc γ ^{-/-} mice that were deficient for Fc ϵ RI failed to do so, demonstrating that Fc ϵ RI-expressing cells other than mast cells are responsible for IgE-CAI. Basophils were the best candidate for these cells, but tools suitable for analyzing basophil functions, including basophil-depleting antibodies and mice deficient only for basophils were not available at that time. Therefore, we performed cell-transfer experiments in which various fractions of bone-marrow cells isolated from wild-type mice were adoptively transferred into Fc γ ^{-/-} mice [24]. The adoptive transfer of the CD49b (DX5)⁺ fraction, which represented 3~4% of the nucleated bone-marrow cells, reconstituted the IgE-CAI response in the recipient mice. On the other hand, the adoptive transfer of wild-type bone-marrow cells devoid of the CD49b⁺ fraction did not confer IgE-CAI on the recipient Fc γ ^{-/-} mice. These results implied that the CD49b⁺ fraction of wild-type bone-marrow cells contained the Fc ϵ RI-expressing cells responsible for IgE-CAI. Although the majority of CD49b⁺ bone-marrow cells were NK1.1⁺Fc ϵ RI⁻ NK cells, as expected, 15-20% were negative for NK1.1 and c-kit, and they expressed Fc ϵ RI on their surface, indicating that they were not NK cells or mast cells. Light microscopic examination demonstrated that the CD49b⁺Fc ϵ RI⁺ cells possessed lobulated and often ring-like nuclei. Giemsa stained almost no granules in their cytoplasm, but electron microscopic examination revealed electron-dense granules, characteristic of and consistent with their identification as basophils. From these findings, we concluded that the CD49b⁺Fc ϵ RI⁺ basophils were responsible for the development of IgE-CAI [24].

Flow cytometric analysis of cells isolated from the IgE-CAI skin lesions demonstrated that, although basophils were indeed recruited to the skin lesions, they accounted for only ~2% of the isolated cells, in sharp contrast to the massive

infiltration of basophils in guinea pig CBH. Eosinophils and neutrophils were the major infiltrates in mouse IgE-CAI. It is extremely difficult to detect basophils in tissue sections by light microscopy, and therefore their identification with electron microscopy has been recommended [4, 5]. To simplify the identification of mouse basophils in tissue sections, we recently established a mAb specific to mouse mast cell protease 8 (mMCP-8), a granzyme B-like serine protease that is selectively expressed by mouse basophils and stored in their secretory granules [48]. The immunohistochemical examination of paraffin-embedded skin samples with this mAb demonstrated the infiltration of a relatively small number of basophils in the dermis at the site of antigen administration. An obvious question raised by this finding was how such a small number of basophils could induce an allergic inflammation associated with a massive infiltration of eosinophils and neutrophils.

Mice deficient only for basophils have not been identified or established yet. Instead, we have developed an anti-CD200R3 mAb, Ba103, that can transiently deplete most of the basophils when administered to mice [25]. CD200R3 is a member of the CD200R family, and functions as an activating receptor in association with an ITAM-containing adaptor molecule, DAP12 [49-52]. Its ligand remains to be identified. The treatment of mice with Ba103 selectively depletes the basophils without affecting the number of mast cells, even though both basophils and mast cells express CD200R3. The Ba103-mediated depletion of basophils before the antigen challenge completely abolished the development of IgE-CAI, confirming the critical role for basophils in IgE-CAI [25]. Moreover, treatment with Ba103 during the course of the dermatitis suppressed the on-going ear swelling and inflammation. Intriguingly, the number of eosinophils and neutrophils infiltrating the skin lesions was drastically

reduced by the treatment, coincident with the ablation of basophils from the inflammation sites [25]. A similar observation was reported in a guinea pig model of tick infestation, in which the depletion of basophils by an anti-basophil serum resulted in a decreased infiltration of eosinophils at the tick feeding site [53]. These results strongly suggested that basophils might function as initiators or propagators rather than as effectors of the allergic inflammation, and that they promote the recruitment of other proinflammatory cells such as eosinophils and neutrophils. The molecules involved in this process remain to be determined. Our preliminary results suggest that antigen/IgE-stimulated basophils secrete soluble factors that include cytokines and proteases that may (Fig. 1, left) act on skin-resident cells such as fibroblasts to produce a large quantity of chemokines. These chemokines may in turn recruit the eosinophils and neutrophils. Future studies are needed to clarify how basophils are recruited to the sites of antigen exposure to initiate IgE-CAI.

It is important to know whether the IgE-CAI-type allergic reaction identified in the mouse model indeed contributes to the pathogenesis of human allergic disorders. Although no definitive evidence for this idea has been acquired to date, several observations suggest that IgE and basophils contribute to human allergic responses. A correlation between the disease severity and serum IgE levels was reported in patients with asthma or atopic dermatitis, particularly in young patients [54, 55]. The beneficial effect of a humanized anti-IgE antibody (Omalizumab) in some asthma patients [56-58] further supports the idea that IgE contributes to the pathogenesis of human chronic allergic disorders. The involvement of basophils in human allergy is less clear, but basophils are often observed in affected tissues in patients with allergic disorders including asthma and atopic dermatitis [14-19], and may contribute to the pathogenesis of chronic urticaria [59, 60].

II. THE ROLE OF BASOPHILS IN SYSTEMIC ANAPHYLAXIS

Anaphylaxis is an acute-onset, potentially fatal, systemic allergic reaction [61, 62]. The phenomenon of anaphylaxis was first described at the beginning of the 20th century. Charles Robert Richet, who received the Nobel Prize in Physiology or Medicine in 1913, and his colleague Paul Portier reported the unexpected fatal reaction in dogs that were immunized with a non-lethal dose of venom from sea anemones and then challenged with a small dose of the venom [63, 64]. They originally intended to tolerate the dogs to the venom by immunizing them with it, on the basis of the discovery of antitoxin (neutralizing antibody against bacterial toxin) by Shibasaburo Kitasato and Emil von Behring. However, the outcome was completely opposite to their intention. Therefore, they designated this curious phenomenon anaphylaxis, which was derived from the Greek words a- (against) and -phylaxis (protection). Kimishige Ishizaka and Teruko Ishizaka dedicated their efforts to elucidating the mechanism for toxin-induced anaphylaxis, and in the mid-1960s they discovered a novel antibody isotype, IgE, as a key element for provoking anaphylaxis [65, 66].

It is well documented that mast cells are critically involved in IgE-mediated systemic anaphylaxis [67-69]. In individuals that have been sensitized to a given allergen and

produce allergen-specific IgE, re-exposure to the same allergen triggers the activation of mast cells through allergen-induced cross-linking of IgE-bound FcεRI on the cell surface, leading to their release of chemical mediators such as histamine. Such mediators act on various cells, including vascular endothelial cells and bronchial smooth muscle, provoking anaphylactic symptoms such as hypotension and dyspnea [67-70]. Basophils can release histamine and leukotriene C4 *in vitro* in response to various stimuli, including FcεRI cross-linking [3, 8]. Therefore, basophils have been considered likely contributors to systemic anaphylaxis. Indeed, basophils are clinically utilized to examine the sensitization status of allergic patients as mast-cell surrogates. Basophils isolated from patients' peripheral blood are incubated with suspected allergens, and their activation is analyzed by the degranulation assay or by flow cytometric analysis using CD203c or CD63 as an activation marker [71]. However, it is uncertain to what extent basophils contribute to systemic anaphylaxis *in vivo*, because they represent only 0.5% of peripheral blood leukocytes and do not usually reside in peripheral tissues, in contrast to mast cells.

Prospective studies of induced anaphylaxis for the purpose of understanding the molecular mechanism of systemic anaphylaxis are generally impractical in human subjects, because of the potential for a rapid, life-threatening reaction. Therefore, systemic anaphylaxis has been studied largely by using animal models. Studies using mouse models demonstrated that active systemic anaphylaxis can be elicited even in mice deficient for either IgE or mast cells [72, 73], indicating that the classical pathway that utilizes mast cells and IgE cannot explain all cases of anaphylaxis, and that an alternative pathway(s) exists [67, 74]. Of note, FcεRI- but not FcγR-deficient mice could exhibit systemic anaphylaxis [75-77]. FcγR-deficient mice fail to express not only FcεRI but also stimulatory IgG receptors. These results strongly suggested that IgG substitutes for IgE in the alternative pathway of systemic anaphylaxis. Indeed, antigen-specific, IgG-mediated systemic anaphylaxis can be elicited in mice that are passively sensitized with antigen-specific IgG, particularly the IgG1 subclass [75, 76]. The low-affinity IgG receptor FcγRIII is largely involved in the IgG-mediated systemic anaphylaxis [77]. Collectively, the current knowledge indicates that at least two pathways can lead to systemic anaphylaxis: the classical one that is mediated by mast cells, IgE, and FcεRI, and the alternative one that is mediated by non-mast cells, IgG, and FcγRIII.

Our finding of a non-redundant role for basophils in IgE-CAI [24, 25] inspired us to explore whether basophils also contribute to systemic anaphylaxis, particularly to the IgG-mediated pathway. For a better understanding of the cellular and molecular mechanism of IgG-mediated systemic anaphylaxis, we established a mouse model of IgG-mediated penicillin anaphylaxis, in which mice are passively sensitized with an intravenous injection of penicillin V (PenV)-specific IgG1 mAb [28]. An intravenous injection of PenV-conjugated bovine serum albumin (PenV-BSA) induced typical anaphylactic manifestations, including a drastic drop (-4~-6°C) in body temperature. Consistent with previous reports using other models, the IgG1-mediated systemic anaphylaxis was induced even in mast cell-deficient mice, although the depression in temperature was slightly less than

in mast cell-sufficient mice. By contrast, IgE-mediated systemic anaphylaxis was completely abolished in mast cell-deficient mice. Thus, mast cells are essential for the IgE-mediated anaphylaxis, but dispensable for the IgG-mediated one.

FcγRIII does not efficiently bind free monomeric IgG, and shows a high affinity for immune complexes composed of IgG and antigens [78], suggesting that cells responsible for the IgG-mediated systemic anaphylaxis should quickly capture, through their FcγRIII, immune complexes that are formed in the circulation soon after antigens are delivered into the bloodstream. Flow cytometric analyses revealed that basophils bind the greatest amount of immune complexes per cell among the cells analyzed, including macrophages and neutrophils, when examined immediately after the antigen challenge [28]. This binding was strongly inhibited in mice treated with anti-FcγRII-III mAb prior to the IgG1 sensitization. These results suggested that basophils are good candidates for cells responsible for IgG-mediated systemic anaphylaxis. Indeed, the Ba103-mediated depletion of basophils before the antigen challenge ameliorated the IgG-mediated systemic anaphylaxis in both mast cell-sufficient and mast cell-deficient mice [28]. On the other hand, mice depleted of macrophages, NK cells, or neutrophils exhibited normal IgG-mediated anaphylaxis in our experimental system. Importantly, the basophil depletion did not ameliorate IgE-mediated anaphylaxis. Collectively, these results indicate that basophils are dispensable for the IgE-mediated anaphylaxis, but play the major role in the IgG-mediated one (Fig. 1, right).

Not only systemic, but also local IgG-mediated anaphylaxis can be induced in mice. Intradermal injection of IgG1 followed by intravenous injection of the corresponding antigens induces a local skin reaction that is called passive cutaneous anaphylaxis (PCA) [79]. IgG-mediated PCA is dependent on FcγRIII [80], like IgG-mediated systemic anaphylaxis, but cannot be elicited in the absence of mast cells [81], in contrast to IgG-mediated systemic anaphylaxis, indicating that mast cells play a critical role in IgG-mediated PCA. In fact, the degranulation of mast cells was observed when they were incubated *ex vivo* with antigen-IgG complexes [80]. In contrast, during IgG-mediated systemic anaphylaxis, their degranulation was rarely detected in peripheral tissues [76]. The differential contribution of mast cells and basophils to local and systemic anaphylaxis, respectively, may be explained by the difference in their anatomical localization and the route of antibody administration. Antibodies are directly delivered into the skin tissue in the PCA model, and thus, tissue-resident mast cells rather than circulating basophils are mainly activated by immune complexes that form within the skin tissue. On the other hand, in the model of passive systemic anaphylaxis, both antibodies and antigens are delivered into the bloodstream, and immune complexes are formed in the circulation, leading to the activation of circulating basophils rather than of tissue-resident mast cells.

In IgE-mediated systemic anaphylaxis, mast cell-derived histamine is the major chemical mediator for inducing anaphylactic symptoms. The treatment of mice with antihistamine showed little or no inhibitory effect on IgG-

mediated anaphylaxis, in contrast to its prominent effect on IgE-mediated anaphylaxis. Of note, an antagonist of platelet-activating factor (PAF) almost completely inhibited the IgG-mediated anaphylaxis [28], whereas it had much less effect on IgE-mediated anaphylaxis than did the anti-histamine treatment. Thus, PAF rather than histamine is the major chemical mediator in IgG-mediated anaphylaxis (Fig. 1, right), in contrast to IgE-mediated anaphylaxis. Although many types of cells, including macrophages and neutrophils, are reported to produce PAF, basophils release much higher amounts of PAF than other cells when stimulated *ex vivo* with allergen-IgG1 immune complexes. PAF released from activated basophils acted on human umbilical vein endothelial cells and induced morphological changes, such as contraction and the loss of reciprocal contact.

These *ex vivo* observations strongly suggested that circulating basophils are stimulated to release PAF through the capture of allergen-IgG immune complexes. The PAF in turn acts on endothelial cells, resulting in increased vascular permeability, and leading to systemic anaphylaxis. However, we wondered if the amount of PAF released from basophils is sufficient to induce anaphylaxis *in vivo*, given that basophils account for only 0.5% of peripheral blood leukocytes. The intravenous injection of histamine or PAF alone induced a drastic drop (-5°C) in body temperature as observed in IgG-mediated systemic anaphylaxis. Notably, the dose necessary for inducing such a temperature drop differed considerably: 3 mg of histamine or 100 ng of PAF. We estimated from *ex vivo* experiments that 100 ng of PAF can be released from 3×10^5 basophils, which is close to the total number of basophils in a mouse [28]. Collectively, these data indicate that basophils can induce systemic anaphylaxis through the release of the potent vasoactive PAF in response to the stimulation of allergen-IgG immune complexes, even though they represent a minor population in the peripheral blood.

Passive systemic anaphylaxis is a simple and convenient model of anaphylaxis, but it may not be relevant to what happens in real life. Therefore, we examined the role of basophils in active systemic anaphylaxis, in which mice are immunized with PenV-conjugated ovalbumin in Alum and *B. pertussis* toxin, and 2 weeks later challenged by an intravenous injection of PenV-BSA [28]. Severe systemic anaphylaxis was induced in both mast cell-sufficient and -deficient mice, and all the mice examined died from anaphylactic shock. In contrast, IgE- or IgG-mediated passive systemic anaphylaxis is not fatal. Ba103-mediated depletion of the basophils before the antigen challenge protected mast cell-deficient mice from death, even though some drop in body temperature was detected, clearly demonstrating that basophils play a critical role in active systemic anaphylaxis as well as in IgG-mediated passive systemic anaphylaxis. Notably, the basophil depletion failed to protect mast cell-sufficient mice from death, indicating that fatal anaphylaxis can be avoided only when both basophils and mast cells are absent. Therefore, both basophils and mast cells appear to contribute critically to active systemic anaphylaxis, most likely via their respective stimulation with IgG- or IgE-immune complexes.

Another study reported an important role for macrophages in a different model of active systemic anaphylaxis,

in which mice were immunized with goat anti-mouse IgD antiserum to induce a large production of IgE and IgG antibodies specific to goat IgG, and subsequently challenged with an intravenous injection of goat IgG [77]. Systemic anaphylaxis was induced even in mast cell-deficient or FcεRI-deficient mice, and it was completely inhibited by the PAF antagonist. The anaphylaxis was prevented, however, in wild-type mice by treating them with a macrophage inactivator, gadolinium chloride, before the antigen challenge, demonstrating that macrophages play a major role in this model system. It is unclear what determines whether basophils or macrophages play the dominant role in active systemic anaphylaxis. Candidates for this role include the genetic background of the mice, the immunization protocol, the Th1/Th2 balance, the nature of the antigen, and the quantities of antigens and antibodies.

For the clinical response to anaphylactic shock, the prompt intramuscular injection of epinephrine is the first choice for treatment, regardless of the underlying mechanism. On the other hand, the ability to distinguish the molecular mechanisms underlying different types of systemic anaphylaxis is essential for risk management and preventing recurrence [62]. It remains to be clarified whether the alternative anaphylaxis pathway mediated by basophils, IgG, and PAF is operative in humans. There is some circumstantial evidence for it, particularly in clinical settings. Several case reports described anaphylaxis that occurred in the absence of detectable allergen-specific IgE in serum or without increased serum levels of tryptase, which is derived from degranulated mast cells [82, 83]. In addition, allergen-specific IgG instead of IgE was reported in individuals who showed systemic anaphylaxis in response to medicines such as protamine, dextran, and recombinant IgG used for antibody therapy [82, 84-86]. Furthermore, human basophils have been shown to release PAF in response to various stimuli [87]. Moreover, a recent clinical study showed a correlation between serum PAF levels and the severity of anaphylaxis [88]. Therefore, the pretreatment of patients at high risk for anaphylaxis with both a PAF antagonist and an antihistamine before administering medications may avert the induction of systemic anaphylaxis.

CONCLUDING REMARKS

The functional significance of basophils has often been questioned, since evidence that basophils and mast cells play distinct roles *in vivo* was lacking until recently. However, as discussed in this review article, recent studies have illuminated at least some of the nonredundant roles played by basophils in allergic responses. We now know that, at least in mouse, basophils are a major player in IgG-mediated systemic anaphylaxis and that they function as initiators rather than effectors in IgE-mediated chronic allergic inflammation, even though they account for less than 1% of peripheral blood leukocytes. It remains to be clarified whether these findings are relevant to the pathogenesis of human allergic disorders. Nevertheless, the therapeutic and preventive effects of treatment with the basophil-depleting antibody on IgE-mediated chronic allergic inflammation and IgG-mediated systemic anaphylaxis, respectively, in mouse mod-

els, suggests that basophils and their products are promising therapeutic targets for allergic disorders.

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