

Recent Developments in Basophil Research: Do Basophils Initiate and Perpetuate Type 2 T-Helper Cell Responses?

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Key Words

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Abstract

Basophils account for only 0.1–1% of all peripheral blood leukocytes. They were considered to be a redundant cell type for a long time. However, several findings show a non-redundant role for basophils in type 2 T-helper cell (Th2) immune responses in helminth infections, allergy and autoimmunity. Both immunoglobulin-E-dependent and -independent pathways have been described to contribute to basophil activation. In addition, several recent studies reported that basophils can function as antigen-presenting cells and are important in the initiation of Th2 immune responses. However, there are also conflicting studies that do not corroborate the importance of basophils in Th2 immune responses. This review discusses the role of basophils in Th2 immune responses in view of these recent findings.

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Introduction

Basophilic granulocytes have been discovered more than a century ago [1], but it took more than 9 decades to demonstrate their direct involvement in allergy [2]. Granulocytes are divided into three subsets: basophilic granulocytes, eosinophilic granulocytes and neutrophilic granulocytes. Basophilic granulocytes circulate in the peripheral blood and account for approximately 0.1–1% of blood leukocytes. They measure 7–10 μm in diameter, have a segmented nucleus and contain metachromatic granules. Basophils share some features with mast cells and have often been considered as minor, and possibly redundant, relatives of mast cells or as blood-circulating precursors of tissue-resident mast cells [3]. Even though basophils differ from mast cells in several aspects (table 1), they are more conveniently isolated (from the blood) than mast cells (from the tissues) and are often used as a surrogate for mast cells [4]. An important immunological role of basophils emerged when immunoglobulin E (IgE)-dependent interleukin (IL)-4 and IL-13 secretion by these cells was discovered (fig. 1) [5–8]. More recently, several studies in mouse models were published that indicate that basophils may act as antigen-presenting cells (APCs). In addition, basophils were shown to be in-

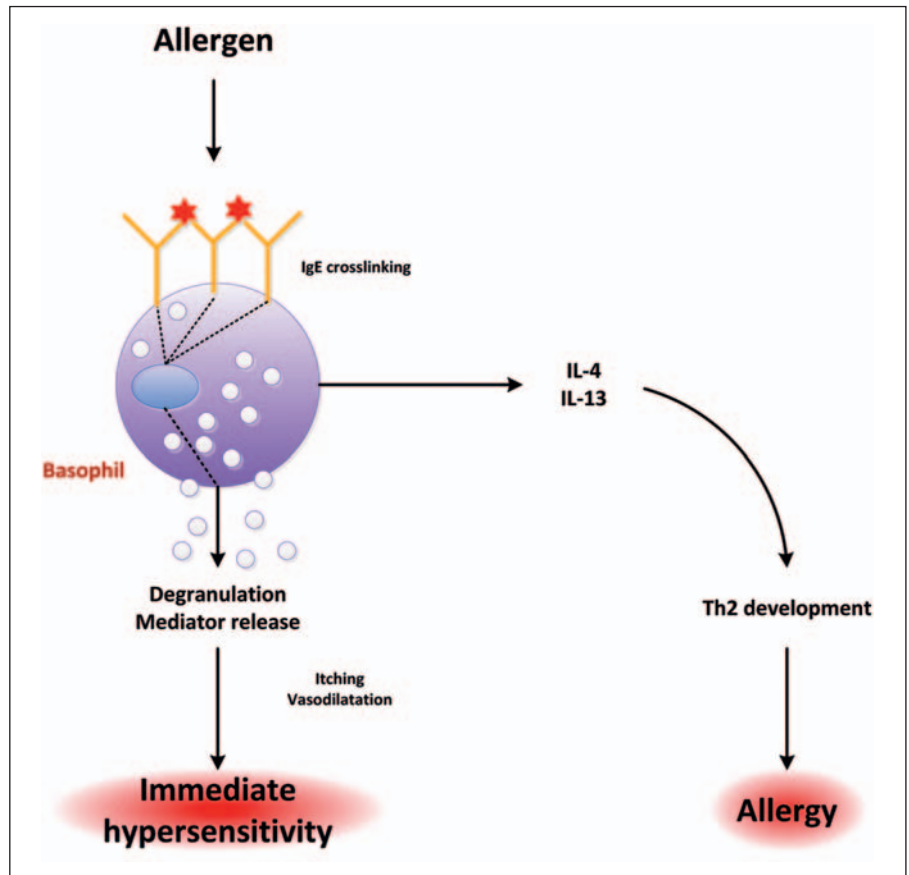


Fig. 1. Classical view of basophil in allergy. IgE crosslinking on the basophil by allergens leads to (1) degranulation and mediator release resulting in immediate hypersensitivity, and (2) to secretion of IL-4 and IL-13 that enhance the Th2 immune response which is involved in allergy.

Table 1. Major features of human mast cells and basophils

	Basophils	Mast cells
Origin	GMP	GMP
Site of maturation	bone marrow	tissue
Lifespan	days to weeks	weeks to months
Primary location	intravascular (<1% of WBC)	tissue
Nucleus	segmented	ovoid
Lipid mediators	LTC ₄ , LTD ₄ , LTE ₄ , PAF	LTB ₄ , LTC ₄ , LTD ₄ , LTE ₄ , PAF, PGD ₂
Granule contents	histamine, chondroitin sulphate, protease Charcot Leyden crystals, MBP	histamine, heparin and/or chondroitin sulphate, protease (trypsin), tryptase
Differentiation and chemotactic factors	IL-3, IL-5, GM-CSF, CCL2, CCL5, CCL7, CCL8, CCL11, CCL13, CCL24, CCL26, leptin, Flt3-L, TSLP	IL-3, IL-4, IL-6, IL-9, IL-33, GM-CSF, NGF, SCF, TSLP, CCL2, CCL5, CCL7, CCL8, CCL11, CCL13, CCL24, CCL26
Secreted cytokines and other growth factors	IL-3, IL-4, IL-5, IL-6, IL-8, IL-9, IL-13, IL-25, APRIL, BAFF, RA, VEGF-A/B, CCL2, CCL3	IL-3, IL-5, IL-6, IL-8, IL-9, IL-13, TGF-β, TNF, TSLP, CCL1, CCL2, CCL3, CCL4, CCL5, CCL8, CCL17, CCL22
Phenotypical markers	FcεRI ⁺ , CD14 ⁻ , CD117 ^{+/-} , CD123 ^{hi} , CD203c ⁺	FcεRI ⁺ , CD14 ⁻ , CD117 ⁺ , CD203c ^{+/-}

GMP = Granulocyte-monocyte progenitors; WBC = white blood cells; LTC₄ = leukotriene C₄; PGD₂ = prostaglandin D₂; MBP = major basic protein; GM-CSF = granulocyte macrophage colony-stimulating factor; Flt3-L = Flt3 ligand; NGF = nerve growth factor; SCF = stem cell factor; APRIL = A proliferation-inducing ligand; RA = retinoic acid; VEGF = vasoendothelial growth factor; TGF-β = transforming growth factor-β; TNF = tumour necrosis factor.

volved in inducing and perpetuating type 2 T-helper cell (Th2) responses. This review describes these recently discovered functions of basophils.

Basophil Progenitors and Differentiation

Human basophils and mast cells arise from CD34⁺ granulocyte-monocyte progenitors in the bone marrow. Differentiation and survival of human basophils is mainly dependent on IL-3, IL-5 and granulocyte macrophage colony-stimulating factor, with IL-3 being 10- to 50-fold more potent than the other two factors [9, 10]. IL-3 also induces ST2 (IL-33R α) expression on basophils, leading to enhanced IL-33 responsiveness [11]. The important role of IL-3 is illustrated by the fact that differentiation of human basophils from human cord blood precursors occurs in 3 weeks in the presence of recombinant IL-3 *in vitro* [12]. Recently, enhanced differentiation, survival and/or activation of basophils have been found under the influence of IL-33 [11] and leptin [13].

Thymic stromal lymphopoietin (TSLP), produced by epithelial cells, stromal cells and mast cells, promotes the expansion of basophils in mice [14–20]. TSLP promotes mouse basophil haematopoiesis and activation independently of IL-3. TSLP-induced basophils are smaller in size than IL-3-stimulated basophils and express higher levels of IL-33R. A role for TSLP in the maturation of human basophils has not been shown to date. However, the majority of basophils from healthy human donors express TSLP receptor. Also, IL-33R levels are significantly higher in human basophils obtained from inflammatory sites, suggesting that TSLP also induces basophil haematopoiesis and activation in allergic humans [14].

Production and Storage of Mediators by Basophils

Basophils produce and store histamine. Upon degranulation, histamine causes symptoms such as flushing, headache and tachycardia, and is involved in the immediate allergic response as well as in anaphylaxis [21]. Basophils express histamine receptors and transporters. Intracellular histamine negatively controls its own synthesis and cytokine synthesis via the organic cation transporter 3 [22]. Besides histamine, several other lipid and protein mediators are stored and secreted by basophils, such as platelet-activating factor (PAF), which is much more potent on a molar basis than histamine [23] and leukotriene C₄ (table 1).

Degranulation of basophils typically occurs upon IgE crosslinking after exposure to allergens. However, basophils can also be induced to degranulate by the complement factors 3a (C3a) and C5a, bacterial peptide N-formyl-methionine-leucine-phenylalanine (fMLP), IgD and cytokines [24–26]. IL-33 alone or in combination with IL-3 enhances IgE-induced histamine release and leukotriene C₄ production, but does not induce degranulation or lipid mediator formation by itself [11]. The release of the preformed mediators causes the symptoms of immediate hypersensitivity [27].

Production of Cytokines

Besides the release of preformed mediators, basophils can also produce several cytokines (fig. 2). They can rapidly produce and secrete IL-4 and IL-13 upon stimulation. This production is faster than normally expected for *de novo* protein synthesis and can be explained by the constitutive presence of low levels of IL-4 and IL-13 transcripts [28, 29]. In addition, human basophils have been found to store CC chemokine ligand 2 (CCL2) [30].

IL-33 synergizes strongly with IL-3 to increase IL-4 production by basophils. IL-33 belongs to the IL-1 family, is mainly expressed by fibroblasts, epithelial cells and endothelial cells and plays a key role in Th2 responses [31, 32]. Combined with IgE crosslinking, IL-33 also enhances histamine and IL-13 release. IL-33 also promotes mast cell- and basophil-driven inflammation and anaphylaxis, due to its ability to activate IgE-dependent and -independent effector responses [33, 34]. IL-33 induces IL-9 production in human basophils, which is even more increased by simultaneous stimulation with IL-3 [35]. Several additional cytokines are produced by human basophils (table 1).

Mouse basophils not only respond to TSLP as described above, but can also produce TSLP [36]. However, it is not clear yet whether human basophils can produce TSLP as well. Both mouse and human basophils produce IL-25 (or IL-17E), which has an important role in the regulation of Th2 memory cells [37]. Together with TSLP and IL-33, IL-25 can condition dendritic cells (DCs) to induce a unique type of inflammatory Th2 cells, which not only produce IL-4, IL-5 and IL-13, but also tumour necrosis factor- α instead of IL-10 [18, 38, 39]. This suggests a role for basophils in chronic allergic diseases, as IL-25 and IL-25R are associated with these diseases [40].

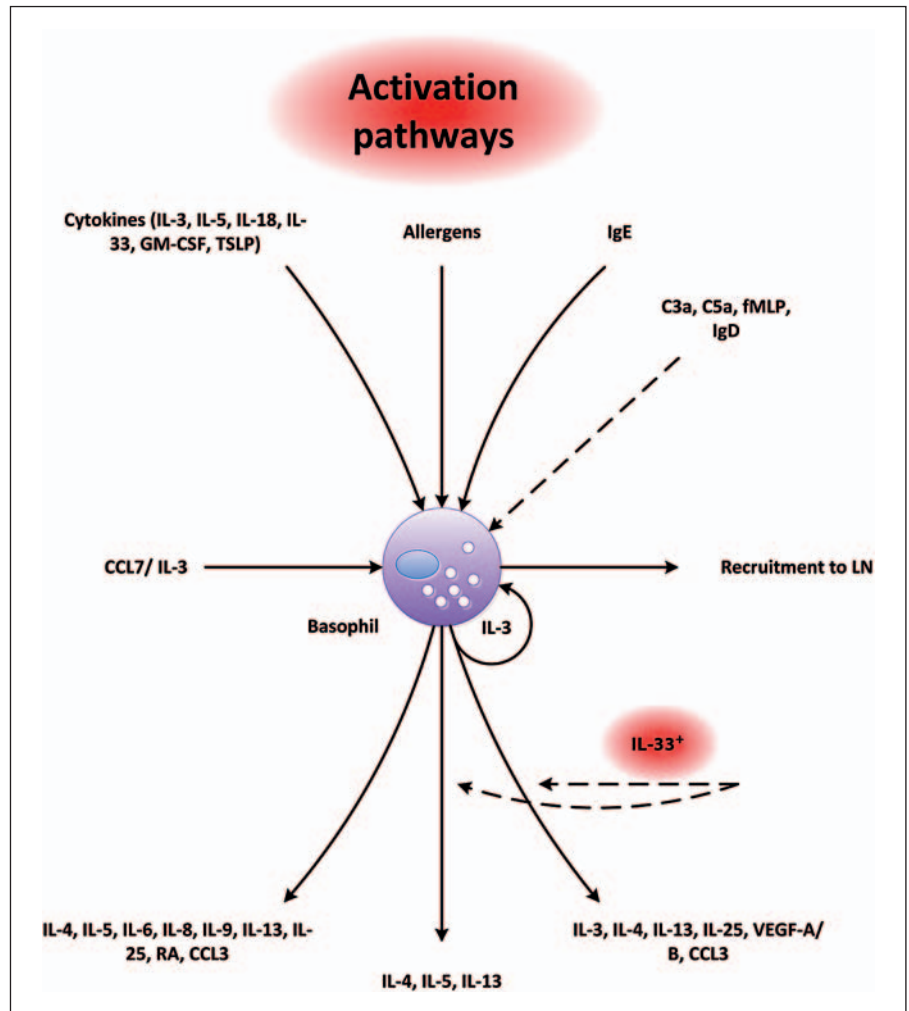


Fig. 2. Activation pathways of basophils. Basophils can be activated by cytokines, allergens and IgE crosslinking. Activation via one of these pathways leads to specific cytokine and chemokine responses. Cytokine responses to allergens or IgE crosslinking can be enhanced by IL-33. Basophils also respond to complement factors C3a and C5a, bacterial peptide fMLP and IgD crosslinking. Furthermore, basophils can be recruited to the lymph nodes by CCL7 and IL-3. GM-CSF = Granulocyte-macrophage colony-stimulating factor; LN = lymph node; RA = retinoic acid; VEGF = vasoendothelial growth factor.

In response to IL-3, human basophils produce retinoic acid, which enhances differentiation of Th2 and regulatory T (Treg) cells and inhibits Th17 cell differentiation [41–43]. Human basophils produce IL-3 upon FcεRI crosslinking, which acts in an autocrine fashion [12]. IL-3-induced production of amphiregulin, which is a strong Th2 stimulus and member of the epidermal growth factor family, has also been found in human basophils [44, 45]. Through FcεRI crosslinking, human basophils also produce vasoendothelial growth factors A and B which are also involved in tissue remodelling [46]. These findings, along with the notion that basophils produce IL-9 [35], suggest a role for basophils in tissue remodelling seen in chronic allergic inflammation [44, 45].

Activation of basophils may also play a role in compromising epithelial barrier function via the production of IL-4 and IL-13. An *in vitro* study in Calu-3 lung epithelial

cells showed a disrupting effect of IL-4 and IL-13 on epithelial barrier function and wound healing. Thus, IL-4 and IL-13 seem to be involved in the exacerbation seen in severe asthma patients [47].

Presence of Basophils at Inflamed Tissue Sites

Basophil infiltrates have been observed in several human allergic diseases, such as atopic dermatitis, allergic asthma and allergic rhinitis [48]. Originally, the involvement of basophils was suggested by the presence of specific mediator profiles in the late allergic responses following allergen provocation [49]. Later, specific antibodies confirmed the presence of basophils in inflamed tissue [50]. Both presence of the basophils and their state of activation indicate a role of basophils in allergic inflammation.

tion, although this has not yet been formally proven. Basophils enter tissue sites within several hours after exposure to allergens [51]. However, it is conceivable that by the time basophils enter these tissues, the allergens may have been cleared already. This evidently leads to the question as to what else, other than allergen-mediated stimulation, can drive basophil activation following extravasation into tissue sites affected by allergic inflammation. Recently, it has been demonstrated that mouse basophils can be activated by IL-18 and IL-33 to release large amounts of cytokines such as IL-4, IL-6, IL-9, IL-13, CCL2, CCL3, CCL4, CCL5 and granulocyte macrophage colony-stimulating factor, but not IL-17, IL-5 and interferon- γ [51–53].

Functional Role of Basophils in Responses to Parasites and in Autoimmunity

For a long time, basophils have been recognized as players in Th2 immunity [2]. In addition to a role in allergy as described above, Th2 responses are important in protective immunity against parasitic infections. Basophils are involved in immunity against parasites such as the intestinal helminths *Trichuris muris* [15], *Necator americanus* [50] and *Nippostrongylus brasiliensis* [29, 54, 55], *Schistosoma mansoni* eggs [15] and ticks [4]. Basophils also protect against the microbes *Moraxella catarrhalis* and *Haemophilus influenzae* by the production of antimicrobial factors via crosslinking of membrane-bound IgD molecules. In addition, IgD crosslinking by bacterial antigen results in support of class switching in B cells from IgM to IgG and IgA by basophil-derived B-cell-activating factor (BAFF) and A proliferation-inducing ligand [56]. Mouse basophils are also involved in supporting plasma cell survival [57], but these findings need to be confirmed in humans.

Besides these crucial roles in Th2 responses in allergy and parasitic infections, basophils are involved in autoimmunity. Autoimmunity is commonly described as a Th1, Th17 and/or Treg cell-mediated response, but several autoimmune diseases are also caused by a predominant Th2 immune response. Basophils have been found to be involved in autoimmune diseases such as autoimmune urticaria [58] and bullous pemphigoid [48], and their IgD-mediated activation could imply involvement in other autoinflammatory diseases [58]. Basophils may also be involved in rheumatoid arthritis, although their role is probably redundant [59].

In systemic lupus erythematosus (SLE), all Th1, Th2, Th17 and Treg cell subsets are involved. Multiple organs

seem to be affected by SLE. Kidney damage (lupus nephritis) by deposition of immune complexes formed by IgG, IgM, IgA or IgE may lead to renal failure and death [60]. Rivera and colleagues [61] used a *Lyn*^{-/-} mouse model for lupus nephritis and showed that basophils play a crucial role in the support of autoreactive plasma cells and the secretion of autoantibodies, as well as the survival and differentiation of B cells, possibly via membrane-bound BAFF and IL-6 secretion. The observation of membrane-bound BAFF expression in mice is similar to what was found in human basophils, in which membrane-bound BAFF is expressed after IgD crosslinking [56, 60, 61]. Furthermore, *Lyn*^{-/-} basophils express more CD62L (L-selectin, important in recruitment to secondary lymphoid tissue), which is dependent on the presence of IL-4 and IgE. In basophils from SLE patients, the activation markers CD62L, CD203c and HLA-DR are up-regulated [61]. Basophils are also detected in lymph nodes and spleens of patients, in contrast to control subjects without SLE. Thus, basophils may be held responsible for the production of autoantibodies in SLE and the perpetuation of the pre-existing loss of B-cell tolerance [61].

Do Basophils Induce Th2 Type Responses?

An increasing number of papers has been published that address the role of basophils in inducing Th2 type responses. At least two major pathways have been identified by which basophils are activated to produce the Th2 signature cytokine IL-4. The IgE-dependent pathway involves the binding of allergen-IgE complexes to Fc ϵ RI, implicating a pre-existing immune response against the antigen, resulting in the formation of allergen-specific IgE antibodies. This raises the question of which cells are involved in inducing this primary IL-4-dependent Th2 response.

Another pathway for basophilic IL-4 production is induced by the presence of cytokines such as IL-3 and IL-33 or, in mice, IL-18 [62]. As discussed above, IL-33 has a pronounced agonistic action with IL-3 on basophils to increase IL-4 and IL-13 release. Interestingly, IL-33 is produced by epithelial cells upon stimulation with allergens or parasitic infection [31, 63] and is thought to be released when epithelial cells are lysed [32]. This may suggest that basophils are triggered to produce IL-4 and IL-13 in response to tissue damage, thus initiating Th2 responses in the absence of pre-formed IgE.

Pathogen-associated molecular patterns such as proteases [62], peptidoglycan and other Toll-like receptor

(TLR) ligands, but not the bacterial peptide fMLP or C5a [12], can also enhance the production of Th2 cytokines by basophils. Upon stimulation with Der p 1, a house dust mite (HDM) protease, or *N. americanus*, human basophils produce high levels of IL-4, IL-5 and IL-13 in an IgE-independent fashion [64]. These IgE-independent activation pathways may point to an important role for basophils in providing the initial IL-4 and IL-13 needed to prime Th2 cells in response to tissue damage or infection.

However, this suggests that basophils should also be able to act as APCs. Professional APCs are very efficient in taking up, processing and presenting antigens to naïve T cells. They provide peptides via major histocompatibility complex (MHC) molecules, they costimulate by molecules such as CD80 and CD86, and they produce cytokines [65].

Studies in mouse models have resulted in insight into the antigen-presenting processes of basophils. Mouse basophils were reported to present antigens to CD4⁺ T cells and to express relevant costimulatory molecules, despite having a very low MHC class II (MHC-II) expression compared to DCs and B cells [57]. Several studies in mouse models have even shown that basophils rather than DCs are the critical APCs or at least the critical providers of IL-4 for the local induction of allergen-specific Th2 type responses [15, 66, 67]. Other studies have added considerable doubt to these findings [68, 69].

One of the first studies that reported the necessity of basophils in inducing Th2 type responses in vivo was a study by Sokol et al. [36]. The authors depleted >90% of the basophils, but no skin or intraperitoneal mast cells by administration of the MAR-1 antibody against FcεRIα. They observed, after papain immunization, that mouse basophils are necessary to induce TSLP-dependent Th2 skewing in the lymph nodes [36]. Furthermore, they showed that basophils produce IL-4, IL-13, TSLP and CCL1 in response to papain stimulation [36]. In a follow-up study, the same group demonstrated that mouse basophils cause Th2 cell differentiation in an MHC-II-dependent and IL-4-dependent manner, both in in vitro and in vivo experiments [66].

Further, Perrigoué et al. [15] showed that when MHC-II expression is restricted to CD11c⁺ cells and no MHC-II is present on, amongst others, basophils, an improper Th2 response against *T. muris* is induced. In IL-4-eGFP (4get) mice, IL-4-producing basophils have been found to respond to *T. muris* infection, expressing MHC-II at an intermediate level [15]. Basophils can also promote the proliferation and production of IL-4 by CD4⁺ T cells

in vitro, which is MHC-II dependent [15]. In another study by Yoshimoto et al. [67], basophils were the only APCs that are able to induce Th2 cells. Contrasting with other APCs, basophils pulsed with 2,4-dinitrophenyl-conjugated ovalbumin (OVA) in the presence of 2,4-dinitrophenyl-specific IgE antibodies have a greater capacity to induce the proliferation of OVA-specific T cells. This can be explained by FcεRI expression on basophils which mediates the effective uptake of allergen-IgE complexes leading to more efficient antigen presentation [67].

These studies clearly indicate that basophils play an important role in the induction of Th2 responses in mice. However, others could not reproduce these results and have found no measurable effects of basophils in mice infected with active *S. mansoni* or eggs after depletion of basophils by MAR-1 antibody to FcεRIα [70]. Instead, 70–80% CD11c⁺ DC depletion in the same system as used by Sokol et al. [36] disrupted Th2 induction. In contrast to the data obtained by Sokol et al. [36], this implies that a key role for basophils in the induction of the Th2 response induced by schistosome eggs may be unlikely [70].

Apart from an inducing role of basophils for Th2 responses, another model can be proposed in which DCs are key APCs, but basophils provide the IL-4 and IL-13 to induce a Th2 response. HDM inhalation results in recruitment of inflammatory DCs, basophils and eosinophils in a TLR4-dependent pathway. Depletion of basophils in this model only partially reduces Th2 responses, but depletion of eosinophils has no effect on Th2 responses. Therefore, a model has been proposed whereby DCs initiate and basophils amplify Th2 immunity to HDM allergen [68].

A study by Tang et al. [71] suggests that both DCs and basophils are needed to generate a Th2 response. Mouse basophils immunized with endogenous or exogenous OVA plus papain are not sufficient to effectively stimulate proliferation of CD4⁺ T cells. Depletion of mouse basophils by injection of MAR-1 antibody does have no effect on T-cell proliferation, but reduces the IL-4 production by CD4⁺ T cells. Furthermore, DCs have been shown to have an essential role in the uptake and presentation of papain and OVA. However, DCs alone are unable to produce sufficient amounts of IL-4 to induce IL-4 production in Th2 cells. Basophils alone are also unable to induce IL-4 production in Th2 cells. The combination of DCs and basophils are required to induce a considerable number of IL-4⁺ Th2 cells. In summary, this study suggests the need of DCs to induce CD4⁺ T-cell proliferation, whereas basophils are mandatory as an accessory cell in

providing IL-4 in response to papain. It has also been found that reactive oxygen species signalling is crucial to trigger TLR4 and the subsequent production of TSLP by epithelial cells, to suppress Th1 cytokine production in DCs and to induce DC-derived CCL7 production that recruits basophils via CCR3 to the lymph node [71]. However, in the mentioned studies using MAR-1 antibodies to deplete basophils, also a subset of inflammatory FcεRI⁺ DCs are depleted. It is therefore not clear whether the observed impairment of Th2 induction is due to basophil or DC depletion [68].

Ohnmacht et al. [72] used transgenic *Mcpt8Cre* mice, which constitutively have only 10% or less basophils compared to normal mice, but have normal mast cell numbers. They concluded that basophils are not required in primary Th2 immunity against *N. brasiliensis*, OVA-alum and papain, and do not prime Th2 cells under these conditions. DCs appear to be the key cells to induce T-cell proliferation and differentiation upon papain challenge [72]. Min and colleagues [73] showed an additional effect of IL-3 on mouse basophils. IL-3 is required for transient recruitment of basophils to the lymph nodes after 3–4 days during infection with *N. brasiliensis*. However, absence of IL-3 does not affect the IL-4 production by CD4⁺ T cells and the Th2 immune response. Therefore, they concluded that basophils may be dispensable for the initiation of Th2 responses in *N. brasiliensis* infection [73]. Basophils are also found to be the major source of IL-4 during primary infection with *N. brasiliensis*, whereas IL-4-producing Th2 cells are the major source of IL-4 during secondary infection [54]. In addition, basophil migration was found to be important in mounting the Th2 response in the primary but not in the secondary infection. However, basophil-derived IL-4 is not required to support Th2 differentiation in primary nor in secondary infection [54].

By imaging the interactions between basophils and CD4⁺ T cells, Sullivan et al. [74] showed that mouse basophils interact only briefly with CD4⁺ T cells in the lymph nodes after immunization with *S. mansoni* eggs or papain plus OVA, but they interact significantly longer with CD4⁺ cells in the lung after infection with *N. brasiliensis* with or without OVA. However, notably, different immunization conditions were applied, which might also have influenced the results.

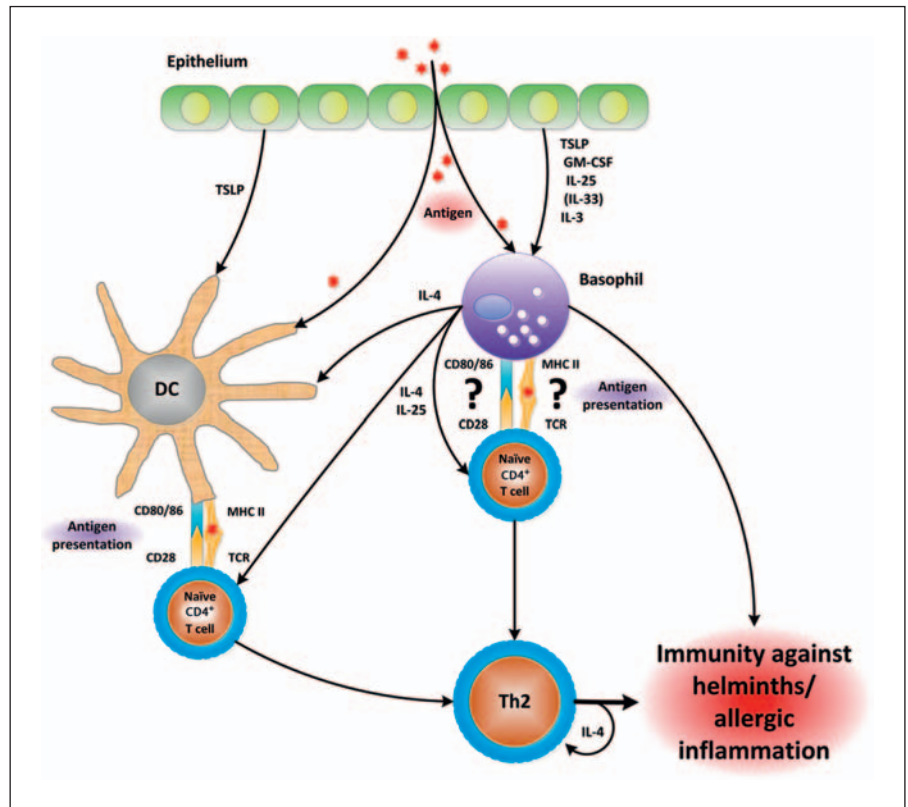
Despite the large number of research efforts, the precise mechanism by which basophils contribute to Th2 responses against pathogens and allergens is not entirely clear yet. It might be concluded that they only have an accessory role in which they provide IL-4 and IL-13 and act

synergistically with DCs. Alternatively, others clearly show that in some models, basophils are the main APCs and provide IL-4 and TSLP as well (fig. 3). The nature of the antigen and the site where the antigen is encountered may play a crucial role in determining whether basophils are the key APCs in inducing and maintaining Th2 responses, or merely are an accessory cell.

Prolongation of Th2 Type Responses by Basophils

As described above, basophils are important in the initiation phase of Th2 responses. This section will discuss evidence that basophils are also involved in prolonging ongoing Th2 responses. Prolongation of the Th2 type response in e.g. allergy by basophils is mediated by IgE crosslinking resulting in histamine and cytokine production. Basophil-depleted mice suffer from increased *N. brasiliensis* burden and impaired worm expulsion compared to non-depleted mice [29]. The role of mast cells and eosinophils in worm expulsion has been excluded by other studies [75, 76]. It has also been shown that basophil depletion leads to reduced eosinophil numbers in blood, spleen and lungs in response to worm infection [29]. Deletion of both basophil-derived and Th2 cell-derived IL-4 and IL-13 results in an increased *N. brasiliensis* burden in mice as well [74]. Papain stimulation and IgE crosslinking induces TSLP expression in mouse basophils. Furthermore, activated basophils have been found to produce TSLP protein in the lymph nodes, which is related to chronic allergic inflammation [36]. Mouse basophils enhance memory responses in vivo. Being the major source of IL-4 and IL-6 in spleen and bone marrow after restimulation with allophycocyanin, basophil depletion leads to an impaired humoral memory response and increased susceptibility to *Streptococcus pneumoniae* [77]. In the *Mcpt8Cre* mouse model, basophils do play an important role in the protective memory response to secondary infection with *N. brasiliensis* and are essential in IgE-mediated chronic allergic inflammation by recruiting eosinophils [72]. The latter finding corroborates the findings in another mouse model, in which basophils were depleted with Ba103 antibody to CD200R3 and have been shown to contribute only in a minor way to IgE-mediated immediate-type allergic reactions. In contrast, basophils are essential initiators of this chronic inflammation, but not in type I hypersensitivity [78]. They found that in another mouse model, protection against secondary infection with *N. brasiliensis* is also critically dependent on basophils [69].

Fig. 3. Integration of current knowledge on basophils. When antigen enters the body, it passes the epithelial barrier, causing tissue damage in several cases. Epithelial cells may be triggered to produce cytokines that prime basophils and DCs. As a result, basophils rapidly produce IL-4, which primes naïve Th cells to differentiate into Th2 cells. Also, IL-4 combined with TSLP activates DCs to prime naïve Th cells to differentiate into Th2 cells. Th2 cells are responsible for protective immunity against helminths and allergic inflammation. DCs are known to interact with Th cells to present antigen and to provide costimulation. In some mouse models, basophils do the same job. The question marks at antigen presentation by basophils in this figure underline the need for data on antigen presentation by human basophils. GM-CSF = Granulocyte-macrophage colony-stimulating factor; TCR = T-cell receptor.



Thus, in addition to the initiating role in Th2 immune responses, basophils are involved in the maintenance and modulation of Th2 immune responses by IgE crosslinking and IL-4 and IL-13 secretion.

Discrepancies between Human and Mouse Basophils

As many studies have been performed on mouse and human basophilic surface markers and functions, several phenotypical as well as functional differences have been observed. Mouse basophils can be characterized by the expression of CD11b, CD49b, CD200R3, FcεRI, Thy1.2 and 2B4 and the absence of CD3, CD117, CD11c, B220, Gr1 and NK1.1 [79]. Human basophils can be characterized by the expression of CD49b, CD123^{hi} (IL-3R), CD192 (CCR2), CD193 (CCR3), CD203c and FcεRI and the absence of CD3, CD11c and CD14 (table 1). They also express several TLRs, such as TLR2 and TLR4 [12, 80]. Furthermore, they bear receptor-bound IgD on their membrane [56].

Mouse basophils induce anaphylaxis by the release of PAF via stimulation of FcγRII-III by IgG-antigen immune complexes [23]. Human basophils do express FcγRII (CD32) [81] and FcγRIIB (CD16b) [82], but seem to lack FcγR-mediated activation due to the presence of FcγRIIB and the coupled immunoreceptor tyrosine-based inhibition motif. In addition, FcγRIIB signaling in mouse basophils seems to differ from human basophils [83]. Furthermore, in contrast to rodents, the existence of an FcγR-mediated anaphylaxis in man remains controversial [84]. Therefore, it is doubtful whether human basophils are involved in FcγR-mediated anaphylaxis as observed in mice [51], although severity of human anaphylaxis is directly correlated with serum PAF levels and inversely correlated with serum PAF acetylhydrolase activity [85]. However, the contribution of PAF production by human mast cells, monocytes and macrophages is unknown. This could mean that an IgG-mediated anaphylactic pathway may exist in humans or that IgG contributes to anaphylaxis severity, but it is unclear whether human basophils or mast cells are involved in such reactions. Additional studies are needed to elucidate this question.

Another important difference between human and mouse basophils is the lack of protease-activated receptor expression by human basophils. This could mean that the activation observed in mouse basophils by HDM [64] or papain extracts [66] is not comparable to the human situation. Additionally, IL-18 fails to activate human basophils, in contrast to mouse basophils [11]. All these differences show that caution should be applied in translating mouse research on basophils to the human situation. Some functions of basophils such as the antigen-presenting function and TSLP production need to be confirmed in humans.

As discussed above, considerable functional differences have been observed between human and mouse basophils, which underlines the need of confirmation of data obtained from mouse studies in man. The role of human basophils in antigen presentation is not yet clear. There seems to be evidence that human basophils may differ from mouse basophils as they do not act as APCs. Using fluorescently labelled Bet v 1, Kitzmüller et al. [86] showed that human basophils efficiently bind the major birch pollen allergen Bet v 1 through IgE-antigen complexes, but do not internalize Bet v 1 and only marginally upregulate HLA-DR, and fail to induce proliferation and cytokine production in Bet v 1-specific T cells. Additionally, Niederberger and colleagues [87] found that basophils of allergic patients are not capable to induce T-cell proliferation in secondary responses to Bet v 1. Various allergen-loaded APCs (DCs, monocytes and macrophages), depleted of basophils, do induce T-cell proliferation. Moreover, adding basophils to these APCs does not have any effect on T-cell proliferation in allergic immune response [87].

Conclusions

An early Th2 skewing function and a potential role in antigen presentation by basophils was discovered recently. Some basophil functions were found to be non-redundant, unique and not shared with mast cells or other immune cells. The findings discussed in this manuscript indicate that basophils modulate the immune system by cytokine (e.g. IL-4 and IL-13) production and are players in Th2 immunity in allergies and against parasitic infections. Mouse basophils can act as APCs, but their role as APC is possibly redundant. Several findings corroborate that mouse basophils act as accessory cell to support DCs in mounting a Th2 immune response, in which DCs act as critical APCs and basophils provide IL-4 (fig. 3). However, most of the data presented so far are generated in mouse models. The first studies to confirm the described mouse basophil functions in man indicate a functional difference between mouse and human basophils. Future studies should focus on confirming important findings on mouse basophils in human basophils to make it possible to draw firm conclusions. In addition, the interaction of basophils with epithelium, DCs and CD4⁺ T cells is incompletely understood. These studies may yield novel therapeutic targets to improve conditions for patients suffering from allergic and autoimmune diseases in which basophils play a major role.

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