



Basophils in inflammation

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ABSTRACT

Basophils are functionally closely related to mast cells. Both cell types express the high-affinity IgE receptor (FcεRI) and rapidly release preformed mediator from intracellular stores upon IgE-mediated activation. However, in contrast to mast cells basophils finish their maturation in the bone marrow and have a lifespan of only 2–3 days. Basophil numbers increase in response to IL-3 or TSLP and migrate into tissues to promote type 2 immune responses. Here we review recent advances regarding the pro- and anti-inflammatory functions of basophils in murine models and human allergic inflammation of the skin, lung and intestine.

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1. Introduction

Inflammatory responses are classically characterized by the presence of swelling (tumor), redness (rubor), heat (calor), pain (dolor) and loss of function (functio laesa). They are evoked by various noxious stimuli including chemical irritants, toxins, physical injury, trauma, and infections. Recruitment of effector cells depends on the site of inflammation and is regulated by inflammatory mediators released from cells present at the site of injury, such as epithelial cells, macrophages, dendritic cells or granulocytes. Thus, a complex network of cells collaborates to counteract the entry of infectious or harmful agents. Parasites generally evoke a so-called type 2 immune response which is characterized by high IgE titers, eosinophilia, basophilia, and increased numbers of IL-4-producing CD4 T cells. However, dysregulation of this network may lead to allergic reactions, like dermatitis, asthma, or food allergy, against otherwise harmless substances. It is still largely unclear which factors induce type 2 immune responses.

Already discovered more than 130 years ago by Paul Ehrlich, basophils have long been ignored due to their rare abundance in the blood (Ehrlich, 1879). In the 1970s it was observed that they can release histamine upon cross-linking of the high-affinity receptor for IgE (FcεRI) (Ishizaka et al., 1972). Only in the 1980s basophils were discovered in mice (Dvorak et al., 1982) and a decade later they were recognized as source of the Th2-associated cytokine IL-4 (Brunner et al., 1993; Seder et al., 1991). With the generation of IL-4-reporter mice it was discovered, that basophils constitutively express IL-4,

facilitating their identification and characterization (Min et al., 2004; Mohrs et al., 2001; Voehringer et al., 2004). Deletion of basophils by monoclonal antibodies revealed many non-redundant functions, some of which were confirmed later with the generation of basophil-deficient mice. To date several mouse models to study basophil function are available and summarized in Box 1. Whether basophils serve as antigen-presenting cells during type 2 immune responses is still highly debated but recent studies indicate that they are dispensable for priming of Th2 cells in most conditions (Eckl-Dorna et al., 2012; Hammad et al., 2010; Kim et al., 2010; Kitzmuller et al., 2012; Nakanishi, 2010; Ohnmacht et al., 2010; Otsuka et al., 2013; Perrigoue et al., 2009; Phythian-Adams et al., 2010; Sharma et al., 2013; Sokol et al., 2009; Yoshimoto et al., 2009). Further studies demonstrated that basophils contribute to the pathogenesis of allergic skin and airway inflammation, and contribute to immunity against parasites both in the gut and the skin (Mukai et al., 2005; Obata et al., 2007; Ohnmacht et al., 2010; Sawaguchi et al., 2012; Wada et al., 2010).

Here, we will highlight recent advances made on the function of basophils when the barrier integrity of skin, lung and gastrointestinal tract is impaired and inflammatory responses unfold (Fig. 1).

2. Role of basophils in tissue inflammation

2.1. Skin inflammation

2.1.1. TSLP-mediated skin inflammation

Early studies with guinea pigs already revealed basophil recruitment to the skin during cutaneous basophil hypersensitivity

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Box 1–Genetically engineered mouse models of basophil-deletion.

Mcpt8 ^{DTR} (Wada et al., 2010)	The human DT receptor was inserted in the 3' UTR of <i>mcpt8</i> . Diphtheria toxin injection depletes more than 90% of basophils within two days.
Mcpt8Cre (Ohnmacht et al., 2010)	BAC-transgenic mice express Cre recombinase under the control of regulatory elements of <i>mcpt8</i> . Cre toxicity constitutively deletes > 90% of basophils.
Basoph8 (Sullivan et al., 2011)	An IRES-YFP-Cre cassette was inserted before the <i>mcpt8</i> start codon. Cross to R-DTA mice constitutively deletes > 90% basophils.
Bas-TRECK (Sawaguchi et al., 2012)	The human DT receptor was inserted under control of the 3' proximal enhancer in the <i>il4</i> locus. DT injection deletes > 90% of basophils.
Runx1 ^{P1N/P1N} (Mukai et al., 2012)	Disruption of the P1 promoter of <i>runx1</i> by the insertion of a neo-cassette leads to constitutive deletion of > 90% basophils.

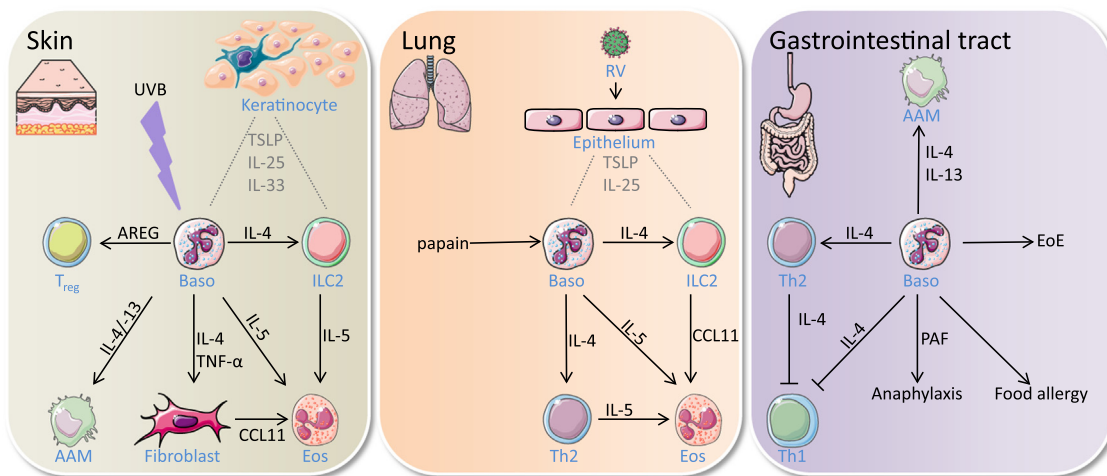


Fig. 1. Illustration of basophil functions during inflammatory response in skin, lung and intestine. Symbols are derived from Servier medical art (Servier, Suresnes, France).

(Dvorak et al., 1970). Basophils were detected in the skin of patients suffering from various skin diseases such as allergic contact dermatitis, atopic dermatitis, prurigo, urticarial, eosinophilic pustular folliculitis and bullous pemphigoid (Dvorak and Mihm, 1972; Ito et al., 2011; Mitchell et al., 1982; Ying et al., 2002). The pathogenesis of these inflammatory conditions is orchestrated by complex interactions between different cell types.

Atopic dermatitis (AD) is characterized by skin inflammation, impaired barrier function, and subsequently leads to an elevated risk of acquiring bacterial infection and allergic sensitization. Reportedly, patients with polymorphisms in the gene encoding thymic stromal lymphopoietin (TSLP) have an increased risk of developing AD and TSLP was detected in skin lesions of AD patients, highlighting the potential role of TSLP in the initiation of AD (Gao et al., 2010). Interestingly, TSLP promotes IL-3-independent basophilia (Siracusa et al., 2011).

One of the most widely used mouse models mimicking AD, involves the topical application of the low-calcemic vitamin D3 analogon MC903 (also named calcipotriol), a treatment regimen for psoriasis (Staberg et al., 1989). TSLP-overexpression in and MC903-triggered TSLP release from keratinocytes leads to leukocyte infiltration and inflammation (Li et al., 2009, 2006). Basophils and group 2 innate lymphoid cells (ILC2s) are among the first infiltrating cells in the skin, though basophils enter the skin before the ILC2s and both cell

types accumulate in close proximity of each other (Kim et al., 2014). Using diphtheria toxin (DT)-mediated depletion of basophils in BasTRECK mice, it was shown that basophils were required for optimal IL-33-independent ILC2 responses. However, others have reported a non-redundant role of IL-25- and IL-33-dependent ILC2s for the pathogenesis of MC903-induced AD (Salimi et al., 2013).

Oposing this model, another report demonstrated that basophils, dendritic cells and CD4 T cells cooperate in order to induce inflammation in the MC903 model (Leyva-Castillo et al., 2013). Leyva-Castillo et al. proposed that TSLP-activated dendritic cells activate T cells via OX40L which then release IL-3 to recruit basophils into draining lymph nodes where basophils release IL-4 to induce Th2 polarization. However, the role of Th2 cells in this model remains unclear since RAG1^{-/-} mice develop full-blown AD (Kim et al., 2013). Epidermal Langerhans cells were also reported to mediate inflammation in the MC903 model via stimulating release of CCL17 and CCL22 from keratinocytes (Elentner et al., 2009). So far, it remains unclear whether basophils respond directly to TSLP and which downstream mediators are released. Therefore, studies with conditional deletion of TSLP and the TSLP receptor in different cell populations should be performed to uncover the cellular interactions during the pathogenesis of AD.

Basophils were shown to recruit eosinophils to inflamed skin. Topical application of croton oil which contains phorbol 12-myristate 13-acetate (PMA) induces irritant chemical dermatitis (ICD) by the

release of various cytokines and inflammatory mediators from cells within the skin. ICD was attenuated in eosinophil-deficient Δ dblGATA mice, but also in wildtype mice treated with inhibitors for reactive oxygen species (ROS) (Nakashima et al., 2014). The authors could show that basophil-derived IL-4 and TNF- α activated skin fibroblasts in vitro to release CCL11/eotaxin-1. Interestingly, basophil-deficient mice showed only a modest attenuation of ICD, suggesting that pathogenesis requires basophil-independent mechanisms. In contrast to a report demonstrating decreased basophil numbers in Δ dblGATA mice, this study observed no reduction of basophils in Δ dblGATA mice (Nei et al., 2013). Nonetheless, this report demonstrates the capability of basophils to recruit effector cells to the site of inflammation.

2.1.2. IgE-mediated chronic allergic inflammation

Chronic idiopathic urticaria clinically presents itself with long-lasting hives with unknown etiology. Furthermore, 50% of patients are unresponsive to treatment with H1-antihistamines. A recent study by Maurer et al. (2013) demonstrated successful treatment of refractory patients with a monoclonal antibody directed against IgE (Omalizumab). Since basophils are activated via engagement of Fc ϵ RI, it seems possible that basophils are mediating chronic urticaria. In fact it has been shown in mice that they are critical mediators in an IgE-dependent model of chronic allergic inflammation of the skin (IgE-CAI). In this model three waves of an ear swelling response can be observed. The first and second waves occur within hours after injection of the antigen and are dependent on mast cells. The third “chronic” wave develops after two days and is abolished in Mcpt8Cre and BasTRECK mice (Mukai et al., 2005; Ohnmacht et al., 2010; Sawaguchi et al., 2012). Interestingly, basophils were also required to impose an anti-inflammatory M2 phenotype upon the recruited inflammatory monocytes/macrophages to limit inflammation in a CCR2-dependent manner. Inflammatory CD115⁺ bone marrow monocytes transferred into DT-treated Mcpt8^{DTR} mice did not acquire an M2-phenotype, a process shown to be dependent on IL-4 or IL-13 (Egawa et al., 2013). This report confirms earlier studies suggesting an immunoregulatory role of basophils via the induction of anti-inflammatory M2 macrophages (Anthony et al., 2011; Ohnmacht and Voehringer, 2009). In summary, basophils can initiate inflammation of the skin, but at the same time execute suppressive measures to limit inflammation.

2.1.3. UVB-induced immune suppression by basophil-derived amphiregulin

UVB-irradiation-induced immune suppression is often used to treat skin diseases like atopic dermatitis. Immune suppression is mediated by regulatory T cells in response to amphiregulin (AREG) acting on the epidermal growth factor receptor on Treg cells (Zaiss et al., 2013). It was demonstrated that AREG^{-/-} mice show no suppressive effect in a model of UVB-irradiation after 2,4-dinitro-1-fluorobenzene-induced cutaneous hypersensitivity (Meulenbroeks et al., 2015). Indeed, basophils were previously shown to express amphiregulin (Qi et al., 2010). Using mixed bone marrow chimeras in which basophils or ILC2s do not produce AREG, it has been recently shown that basophils, not ILC2s, induce UVB-induced immune suppression (Meulenbroeks et al., 2015).

In conclusion, basophils execute, depending on the model system used, important pro-inflammatory and immunomodulatory functions within the skin. Future studies will hopefully reveal which mediators are critically required to recruit basophils into the skin and how basophils regulate the activity of other cell types.

2.2. Lung inflammation

2.2.1. Basophil response to papain

Basophils are increased in lungs of asthmatic patients and were detected in the lungs of fatal asthma patients (Kepley et al., 2001;

Macfarlane et al., 2000). Asthma is associated with excessive expansion of Th2 cells secreting IL-4, IL-5, IL-9, and IL-13. Furthermore, TSLP has been shown to drive progression from AD to asthma (Han et al., 2012). TSLP produced in the lungs of house dust mite-exposed animals induced basophilia in mice (Siracusa et al., 2011). Several mouse models were developed that induce a Th2-biased lung inflammation.

Basophils were reported to induce Th2 polarization in response to the protease allergen papain. However, we and others have shown that basophils are dispensable for Th2 polarization. ILC2s were shown to drive airway inflammation in response to intranasal challenge with papain (Halim et al., 2012, 2014). Recently, work published by Motomura et al. (2014) demonstrated that basophil-derived IL-4 promotes papain-induced lung inflammation by activation of ILC2s within the lung to recruit eosinophils. Furthermore, basophil-derived IL-4 induced the expression of CCL11/eotaxin-1 in lung ILC2s. Papain can directly activate basophils to produce IL-4. A recent publication characterized the downstream signaling pathways in basophils following papain stimulation. In vitro studies showed that papain-induced IL-4 release depends on the presence of the FcR gamma chain and IL-3 (Rosenstein et al., 2014).

2.2.2. Controversial role of basophils in allergic lung inflammation

In an OVA- or HDM-induced model of airway hyperresponsiveness basophils were shown to be dispensable for Th2 polarization and effector cell recruitment (Hammad et al., 2010; Ohnmacht et al., 2010; Sawaguchi et al., 2012). Recently a study by Zhong et al. reported contradicting data indicating that basophils are primary inducers of Th2 responses in the OVA model. Antibody-mediated depletion of basophils attenuated Th2 polarization and airway inflammation. Furthermore the authors demonstrated antigen uptake and processing by lung basophils, which stands in sharp contrast to several other studies showing the incapacity of basophils to process antigen (Hammad et al., 2010; Otsuka et al., 2013; Zhong et al., 2014).

Asthmatic patients often experience severe exacerbations in response to respiratory viruses. A recent study demonstrated increased recruitment of basophils, eosinophils, neutrophils and Th2 cells in response to rhinovirus-induced and IL-25-mediated activation of ILC2s (Beale et al., 2014). Another group reported that, in atopic asthmatics, the infection with human rhinovirus 16 promoted enhanced IgE responsiveness of basophils three weeks after infection (Agrawal et al., 2014). Their results suggest that basophils are able to promote or prolong rhinovirus-induced lung inflammation during asthma.

IL-25 and ILC2s have also been shown to play an important role during pulmonary fibrosis. The injection of Schistosoma eggs, which get trapped within the lung, is often used to model pulmonary fibrosis. However, contradicting data exist concerning the role of basophils during the development of these granulomas (Anyan et al., 2013; Hams et al., 2014; Schwartz et al., 2014).

2.3. Basophils in gastrointestinal inflammation

2.3.1. Anaphylaxis by food allergens

Food allergies affect more than 17 million Europeans and 15 million people in the US. The symptoms range from skin manifestations, pulmonary stress, gastrointestinal disorders to life-threatening anaphylaxis. Anaphylaxis has been reported to be mediated by three mechanisms: IgE-mediated anaphylaxis and histamine-release by mast cells, IgG1-mediated PAF-release by basophils and/or macrophages and IgG2-Fc γ RIV interaction by neutrophils (Jonsson et al., 2011; Strait et al., 2002; Tsujimura et al., 2008). Yet, the relative contribution of each of the pathways remains to be determined. One of the most important anaphylaxis-causing food allergens are

peanuts. Recently it was shown that both mast cells and basophils contribute to anaphylactic shock in a model of peanut-induced anaphylaxis (Reber et al., 2013). Mice deficient in either mast cells, basophils or both showed a significant but not complete reduction of the immediate anaphylactic hypothermia, serum histamine and PAF levels, indicating that other mechanisms are involved as well. Interestingly, neutrophil depletion did not alter peanut-induced anaphylaxis, although they may be involved in other models of anaphylaxis.

2.3.2. Connection between skin sensitization and gastrointestinal allergic reaction

Moreover, skin sensitization of a given food allergen before oral encounter may lead to the development of food allergy (dual-allergen-exposure hypothesis). Muto et al. (2014) sensitized mice epicutaneously with OVA on SDS-pretreated skin and challenged intragastrically to induce mild anaphylaxis. In this context, basophils were recruited to the skin-draining inguinal lymph nodes and antibody-mediated depletion before epicutaneous sensitization protected from anaphylaxis as were TSLP receptor- and IL-33-deficient mice, suggesting a reinforcing role of basophil-derived cytokines in the priming against allergens. Recent reports implicated TSLP as promoter of atopic march, such as triggering progression from AD to asthma (Han et al., 2012). Now it has also been shown that mice overexpressing TSLP in the skin develop gastrointestinal symptoms of allergy (Han et al., 2014). Moreover, basophil depletion via antibodies and DT in Mcpt8^{DTT} mice attenuated food allergy, although it remains unclear whether basophils promote development of food allergy, act as effector cells, or both. In two studies by Noti et al. (2014, 2013) mice were sensitized with allergens on MC903-induced skin lesions. Intragastric challenge led to TSLP- and IgE-dependent intestinal inflammation, but also to the development of IgE-independent eosinophilic esophagitis. DT-mediated depletion of basophils reduced the allergy score and transfer of basophils was sufficient to promote intestinal inflammation.

2.3.3. Role of basophils in eosinophilic esophagitis

Another food allergy-induced manifestation is the so-called eosinophilic esophagitis (EoE). Triggered by allergens, EoE is characterized by chronic infiltration of eosinophils, mast cells, basophils and Th2 cells to the esophagus. Single nucleotide polymorphisms in the TSLP, TSLP receptor and eotaxin-3 genes were associated with an increased risk of developing EoE (Blanchard et al., 2006; Rothenberg et al., 2010; Sherrill et al., 2010). Elevated TSLP and exaggerated basophil responses in EoE patients have been reported (Siracusa et al., 2011). Basophil depletion before EoE induction was shown to reduce eosinophil infiltration. Neutralization of TSLP or basophil-depletion during EoE ameliorated the disease.

In summary, these studies provide evidence that basophils are involved in the pathogenesis and effector phase of skin-sensitized food allergies, yet the mechanism through which basophils execute their pro-inflammatory role remain unclear. Although basophils do not secrete CCL26/eotaxin-3, basophil-derived IL-4/IL-13 may stimulate CCL26-release by esophageal epithelial cells as it has been shown for CCL11/eotaxin-1 released by fibroblasts during ICD.

In contrast to promoting Th2-driven allergic responses, basophil-derived IL-4 was shown to limit disease activity in a model of murine colitis (Gomez et al., 2014). Adoptive transfer of naive CD4 T cells in T cell deficient RAG^{-/-} or SCID mice leads to the expansion of pro-inflammatory Th1 cells producing IFN- γ and TNF- α that promote colitis. Depletion of basophils, IL-4 or IL-6 further aggravated experimental colitis. Interestingly, a single injection of basophil-depleting antibody before T cell transfer sustainably increased the Th1 response, suggesting a role for basophil-derived IL-4 for the epigenetic modification of T cells during the initial proliferation/polarization. Furthermore, a study by our group demonstrated that basophil-derived IL-4

promoted the early polarization of Th2 cells and the recruitment of alternatively activated macrophages to the small intestine upon secondary infection with the helminth *Heligmosomoides polygyrus* (Schwartz et al., 2014).

3. Conclusion

In summary, basophils are clearly able to drive pro-inflammatory responses by recruiting effector cells such as Th2 cells, ILC2s, eosinophils and inflammatory macrophages to the site of inflammation. At the same time they are able to limit inflammation by release of amphiregulin, induction of alternative activation of macrophages and orchestration of an anti-inflammatory Th2 milieu. However, initiation or augmentation of a type 2 immune response may be beneficial in the context of parasite infection and IBD, but in the context of allergic inflammation may lead to exacerbations and fibrosis. With the now widely available genetically engineered mice basophil research has taken an important step to elucidate the pivotal roles of this rare granulocyte population. Future studies should therefore focus on the molecular mechanisms involved in basophil migration, their interaction with other cell types and their activation to provide new therapeutic intervention strategies to treat allergic disorders.

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