

Basophils: Important emerging players in allergic and anti-parasite responses

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Basophils constitute a subset of granulocytes first described by Ehrlich [1], and named after their property to stain with basic aniline dyes. They are functionally and phenotypically closely related to mast cells, but they clearly represent a separate cell lineage and not, as initially thought, a mast cell precursor population. Mast cells and basophils are clearly distinguishable by flow cytometry and histology using a set of different markers (Table 1). Basophils can rapidly increase in numbers, accumulate in tissues and release cytokines and other mediators during allergic inflammation or parasitic worm (helminth) infection (Table 1). Allergens and helminths have in common that they induce a stereotypic immune response characterized by elevated immunoglobulin E (IgE) levels and increased numbers of eosinophils, mast cells and T helper type 2 (Th2) cells. Despite the well-established association of basophils with such type 2 immune responses, their effector functions remained obscure. This was largely due to limitations for detecting and deleting these rare cells *in vivo*. However, basophils were found to constitutively express interleukin-4 (IL-4) mRNA and,

therefore, IL-4/eGFP reporter mice proved very useful in clearly identifying, isolating and characterizing basophils [2, 3]. With the development of antibody-mediated depletion models, basophils were recognized to be involved at many steps during early and late stages of immune responses against allergens, helminths or autoantigens. MAR-1, an antibody against the high-affinity receptor for IgE (FcεRI) or Ba103, specific for the activating receptor CD200R3, have mainly been used to transiently deplete basophils in mice [4–6]. The caveats of this approach are that in addition to the depletion of basophils both antibodies can activate mast cells and induce anaphylaxis, complicating interpretations of these experiments. This problem can now be avoided by using genetically basophil-depleted mice.

Last year two transgenic mouse strains with either constitutive or inducible depletion of basophils were reported. We generated one of these strains, Mcpt8Cre mice, by random genomic integration of a modified bacterial artificial chromosome that expresses the Cre recombinase under control of regulatory elements of the basophil-specific mast

cell protease 8 [7]. Basophils are spontaneously and constitutively depleted in these mice with high efficiency (>90%) due to “Cre toxicity” caused by high expression levels of the Cre recombinase, which cuts genomic DNA at cryptic loxP sites [8]. Independently, Karasuyama and colleagues generated an inducible strain (Mcpt8^{DTR} mice) by homologous recombination of a diphtheria toxin receptor (DTR)-encoding cassette into the genomic *mcpt8* locus [9]. In these mice basophils can be transiently depleted for a few days by DT injection. Clearly, both mouse strains are valuable tools to further define *in vivo* functions of basophils.

Basophils promote helper T cell differentiation into type 2 (Th2) cells in the context of allergy and parasite infections

Recent studies questioned the role of dendritic cells (DCs) in the initiation of helper T cell differentiation from naïve to Th2 cells, and proposed that basophils are the major cell type inducing Th2 responses. Other studies contradicted these observations, and it seems that basophils can contribute to Th2 polarization but they are probably not essential for this process.

The physiological mechanisms leading to a Th2-polarized immune response against allergens or parasites is poorly understood. IL-4 appears to be an essential factor for Th2 development at least under *in vitro* culture conditions. Therefore, it was postulated that innate cells must exist that provide IL-4 early

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Abbreviations:

APC, antigen-presenting cell; CD, cluster of differentiation; DCs, dendritic cells; IL, interleukin; Ig, immunoglobulin.

Table 1. Summary of markers expressed by murine basophils and/or mast cells and other cells, which can be used for flow cytometry (FACS) or histological stainings

Markers for histology or FACS	Basophils	Mast cells	Other cells
CD49b	+	–	Eo, NK
CD90.2 (Thy1.2)	+	–	T, NKT, neurons
CD117 (c-kit)	–	+	HSC
CD200R3	+	+	None
CD244.1 (2B4)	+	+	NK, T subset
FcεRI	+	+	DC subset
Surface IgE	+	+	B
IL-3R	+	+	M, DC
IL-33R	+	+	Th2
MMCP-1	–	+	None
MMCP-8	+	–	None
Mediators	Basophils	Mast cells	
IL-4	++	+	
IL-5	+	+	
IL-6	+	+	
IL-13	+	+	
TSLP	+	–	
Heparin	–	+	
Histamine	+	++	
PAF	++	+	
Granzyme B	+	–	
β-Hexosaminidase	+	+	

The lower part includes a selection of mediators released by basophils and mast cells.

–, not expressed; +, expressed; ++, highly expressed. Eo, eosinophils; NK, natural killer cells; NKT, natural killer T cells; HSC, hematopoietic stem cells; DC, dendritic cells; M, macrophages; FcεRI, high-affinity receptor for IgE; MMCP, mouse mast cell protease (encoded by *Mcpt* genes); TSLP, thymic stromal lymphopoietin; PAF, platelet-activating factor; FACS, fluorescence-assisted cell sorting.

during initiation of a Th2 response. Evidence that basophils might fulfill this proposed function came from in vitro experiments in which activated basophils promoted the polarization of Th2 cells in the presence of DCs [10]. Other studies showed that basophils can be recruited to lymph nodes [4, 7, 11–13]. Furthermore, basophils were found to take up antigen, express low levels of MHC class II and form conjugates with CD4⁺ T cells [12, 14, 15]. Depletion of basophils with MAR-1 abolished the papain-induced Th2 response, while selective depletion of CD11c⁺ DCs had no effect [4]. Taken together, these studies questioned the role of DCs for initiation of a Th2 response, and proposed that basophils play an essential

role both as antigen-presenting cells (APCs) and early source of IL-4 for Th2 responses induced by papain, *Trichuris muris*, *Schistosoma mansoni* eggs and potentially other allergens and parasites.

However, another study has shown that papain-induced Th2 differentiation requires cooperation between DCs and basophils [16]. In vivo depletion of DCs reduced the Th2 polarization against *S. mansoni* eggs, while depletion of basophils had no effect [17]. Interestingly, a subset of inflammatory DCs in the lung was also depleted by MAR-1 injection since they express FcεRI, and these cells rather than basophils appear critical for Th2 polarization induced by house dust mite inhalation [13]. The same study questioned the role of basophils as

APCs since highly purified basophils neither expressed critical components of the machinery required for antigen presentation nor induced proliferation of antigen-specific CD4⁺ T cells. In constitutively basophil-depleted *Mcpt8Cre* mice, Th2 polarization induced by papain, ovalbumin (OVA) and *Nippostrongylus brasiliensis* appeared normal [7]. So far, the efficiency of allergen or helminth-induced Th2 polarization in *Mcpt8^{DTR}* mice has not been reported.

Currently, it appears that basophils may contribute to Th2 polarization under specific conditions but recent evidence suggests that DCs are probably much more important (reviewed in [18]).

Basophils cause allergic inflammation of the skin

Basophils appear essential for IgE-mediated chronic allergic inflammation (IgE-CAI) of the skin in mice. They also were described to be important for a severe form of system allergic reaction (anaphylaxis), but these results could not be confirmed with basophil-deficient *Mcpt8Cre* mice.

IgE-CAI is elicited by basophils independently of mast cells and Th2 cells as shown by adoptive transfer experiments or antibody-mediated basophil depletion [6, 19]. We recently confirmed the critical role of basophils in this process using the *Mcpt8Cre* mouse model [7]. Interestingly, basophils constitute only 1–2% of the cellular infiltrate, suggesting that their function in this setting is to orchestrate recruitment of effector cells to the skin, although the factors that mediate this process remain to be identified. Therefore, future studies should clarify the potential role of basophils in human inflammatory skin diseases like chronic urticaria and atopic dermatitis.

In addition to their role as orchestrators of IgE-CAI, basophils have been proposed to mediate anaphylaxis, a severe form of allergic reaction that can be elicited by two distinct pathways – at least in mice. The classical pathway is caused by allergens that cross-link IgE bound to FcεRI on mast cells. This results in the release of histamine and lipid mediators, leading to a rapid drop of blood pressure and body temperature. The other pathway is mast cell independent and medi-

ated by release of the vasodilator platelet-activating factor (PAF) after cross-linking of IgG1 bound to Fc γ RIII. Antibody-mediated basophil depletion prevented the release of PAF and protected against anaphylaxis [20]. However, we found no major role for basophils in this process when we performed similar experiments with constitutively basophil-deficient Mcpt8Cre mice [7]. Our results appear more consistent with an earlier study by Finkelman and colleagues, who proposed that macrophages are the main effectors for IgG1-mediated anaphylaxis [21]. To clarify the discrepancy it might be worth repeating the experiment after ablation of basophils in DT-treated Mcpt8^{DTR} mice or selectively deleting Fc γ RIII from macrophages or basophils using conditional knockout mice.

Basophils mediate protective immunity against parasites

It is well known that parasitic worms (helminths) and ticks can induce basophilia, although it remained unclear whether basophils are actively involved in the immune response. Recent work has shown that basophils can indeed contribute to protective immunity against worms and ticks, although it still remains unclear how exactly basophils are involved in this process.

Helminth infections generally induce strong type 2 immune responses characterized by high IgE levels and increased numbers of eosinophils, basophils, mast cells, and Th2 cells. The gastrointestinal nematode *N. brasiliensis* is a natural pathogen of rodents [22]. The worms are expelled by day 8–10 after primary infection by a process that appears to be independent of basophils, eosinophils, and B cells, but requires CD4⁺ T cells and innate IL-4/IL-13-expressing cells [7, 23–25]. The source of the innate IL-4 and IL-13 remains unclear, although mast cells or nuocytes, a recently described IL-13-expressing cell type, seem important for efficient worm expulsion during primary infection [26, 27].

Interestingly, worm clearance after re-infection with *N. brasiliensis* occurs more rapidly and independently of mast cells and CD4⁺ T cells. Serum from

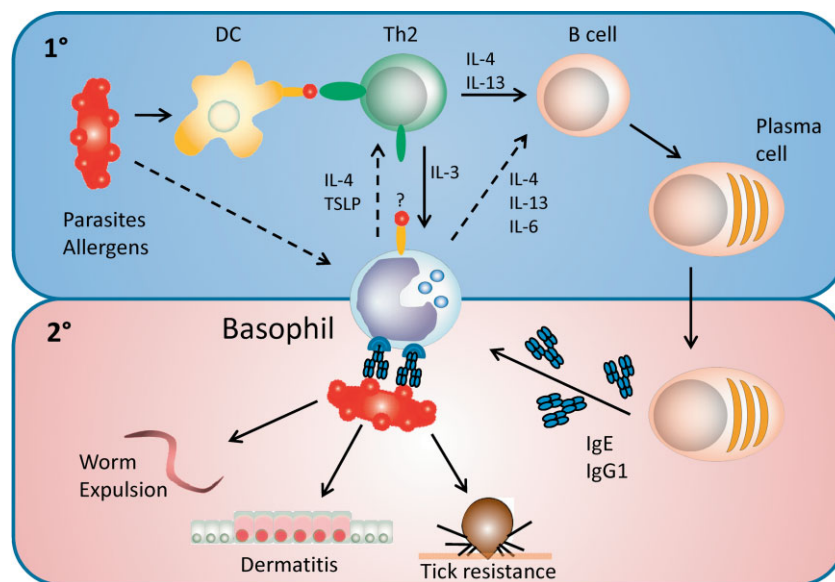


Figure 1. Basophils during initiation and execution of type 2 immune responses. After the first contact (1°) with allergens or parasites, dendritic cells (DC) induce Th2 polarization via MHC class II-T cell receptor interaction and the appropriate environment. Basophils may also serve as antigen-presenting cells (APC) and promote Th2 polarization by release of IL-4 or thymic stromal lymphopoietin (TSLP), although further investigations are required to clarify the in vivo relevance. Th2 cells promote B cell maturation and antibody class switching to IgG1 and IgE by release of IL-4 and/or IL-13. In addition, they mobilize basophils by secretion of IL-3. Basophils may further contribute to B cell activation and class switch recombination. After the primary immune response has subsided, long-lived plasma cells remain in the body and constantly produce low amounts of antigen-specific IgE and IgG1, which sensitize basophils by binding to Fc receptors. During the secondary response (2°) cross-linking of these receptors by the antigen activates basophils, leading to downstream effects like worm expulsion, tick resistance, and allergic inflammation.

N. brasiliensis-immune animals protects naïve animals against infection, suggesting that antibodies can provide protective immunity. Inefficient worm expulsion was observed in re-infected Mcpt8Cre and basophil-depleted mice. Since basophils have a lifespan of about 60 hours, it seems unlikely that the protective memory response is provided by basophils that persisted after the first infection. We rather believe that parasite-specific antibodies are constantly generated by long-lived plasma cells, and these antibodies sensitize newly generated basophils so that they can quickly respond after re-infection (Fig. 1). The protective role of antibodies has also been described in other helminth infection models, and other Fc receptor-expressing cell types like macrophages, eosinophils or neutrophils could be involved in execution of antibody-dependent effector functions [28].

Confirming earlier studies with basophil-depleted guinea pigs, Karas-

uyama and colleagues demonstrated the protective role of basophils against tick infections by depletion of basophils in Mcpt8^{DTR} mice [9]. They showed that basophils were recruited to the infection site, and that protective immunity was dependent on mast cells, antibodies and expression of activating Fc receptors on basophils. Probably tick-specific antibodies bind to Fc receptors on basophils and activate them upon antigen recognition. These findings, together with the above-described function of basophils in chronic allergic dermatitis, highlight their important role as effector cells in the skin and form the basis for future research.

Current understanding of basophil in vivo functions

Depending on the experimental models used, major controversies still exist with regard to the role of basophils as

initiators and executors of type 2 immune responses. Increasing evidence shows that basophils are probably not so critical for the early steps of Th2 polarization and IgE- or IgG1-mediated anaphylaxis. However, they are important for chronic allergic reactions in the skin and contribute to protective immunity against ticks and helminths.

Future directions of basophil research

More than 130 years after the discovery of basophils by Paul Ehrlich we are just beginning to uncover the roles of this enigmatic cell type. The recently generated basophil-deficient mouse models will allow us to further explore the in vivo functions of basophils in the near future. Mixed bone marrow chimeras with Mcpt8Cre mice and a variety of knockout mice can now be generated so that basophils with defined genetic modifications can be studied in vivo. The Mcpt8^{DTR} mice will be useful to deplete basophils during different stages of an immune response and, thereby, help in figuring out when exactly basophils are required for primary or secondary immune responses. Using these novel tools we will gain further insight into the in vivo functions of basophils. Obviously, it is important to determine whether the recent findings that were obtained with mouse models can be applied to the human immune system. A better understanding of the mechanisms that regulate plasma cell survival and basophil activation, homeostasis and recruitment to sites of inflammation will hopefully lead to the development of new therapeutic strategies for allergic disorders and protection against pathogens.

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