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Basophils as APC in Th2 response in allergic inflammation and parasite infection

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Basophils are important effector cells, which contribute to protection against helminths and execute proinflammatory effector function during allergic inflammation. Basophils are also regulators of Th2 responses in helminth-infected hosts and in allergen-injected animals. Recently, three groups using different experimental systems have shown that basophils are antigen-presenting cells (APC), which induce Th2 cells both *in vitro* and *in vivo*. Basophils express MHC class II and CD80/86, have the potential to take-up and process protein antigen (Ag), particularly Ag-IgE complexes, and to present peptide with MHC class II and produce IL-4. However, relevance of basophils as Th2 cell-inducing APC *in vivo* has been challenged by several recent reports that favor the concept that basophils and DC cooperate or basophils merely amplify DC-driven Th2 cell differentiation. In this review, I summarize and discuss the data on the role of basophils as Th2 cell-inducing APC in allergy and parasite infection.

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Introduction

Mast cells, basophils, and eosinophils are important effector cells in helminth infection and allergic inflammation [1,2,3[•],4[•],5]. Basophils are rare circulating cells, accounting for less than 1% of total circulating granulocytes. Basophils, mast cells, and eosinophils arise from the same progenitor. Basophils and eosinophils complete their terminal differentiation in bone marrow. By contrast, mast cells migrate as immature cells from the bone marrow to the peripheral tissues, where they undergo their terminal differentiation [1,2]. Mast cells that complete their differentiation in the skin or in the intestine develop into connective tissue mast cells (CTMCs) and mucosal mast cells (MMC), respectively. Circulating basophils share

several features with tissue-resident mast cells. Both cell types constitutively express FcεR1 and contain basophilic granules in the cytoplasm, and upon cross-linking of FcεR1-bound IgE with multivalent antigens, immediately release various kinds of effector molecules such as histamine and lipid mediators, and Th2-associated cytokines such as IL-4, IL-5, and IL-13, causing immediate type hypersensitivity [1,2,3[•],4[•],5]. Eosinophils also express FcεR1 and induce allergic inflammation by production of chemical mediators when stimulated with cross-linking of FcεR1-bound IgE with antigen. Owing to scarcity of cell number, paucity of specific basophil markers, and their functional similarity to mast cells, basophils have been simply regarded as effector cells of the Th2 immune response [3[•],4[•],5].

IL-3 is a growth factor for basophils. Furthermore, IL-3 is required for optimal basophil IL-4 and IL-13 production. FcR common γ-chain (FcRγ) is a widely expressed adapter, which bears an immunoreceptor tyrosine-based activation motif (ITAM) [6]. This adapter protein is known to associate with various FcR including FcεR1. Recent study by Hida and colleagues demonstrated that basophils lacking FcRγ could proliferate normally but failed to produce IL-4 in response to IL-3, suggesting that FcRγ-mediated IL-3 signal is crucially involved in induction of IL-4 production by basophils [7]. Now it is well recognized that basophils strongly produce IL-4 and IL-13 when stimulated with cross-linking of FcεR1 and IL-3.

The effect of IL-3 is also found in IgE-independent basophil IL-4 production [5,8,9[•]]. Basophils express IL-18R and IL-33R and very strongly produce IL-4 and IL-13 when stimulated with IL-18 or IL-33 in the presence of IL-3 *in vitro* [8,9[•]]. Basophils also express Toll-like receptor (TLR)1, TLR2, TLR4, and TLR6 and produce Th2 cytokines in response to IL-3 plus corresponding TLR ligands [10]. Thus, there are at least two major activation pathways for basophil IL-4 production. One is the Ag/IgE complex-induced activation pathway responsible for ‘acquired-type allergic inflammation’ and the other is the IL-18, IL-33, or pathogen associated molecular pattern (PAMP)-induced activation pathway responsible for ‘innate type allergic inflammation’. Here, I review the major function of basophils as effectors cells in the development of allergic inflammation and the recently found novel function of basophils as Th2 cell-inducing APC in allergy and helminth infection.

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Development of basophils, mast cells and eosinophils

Blood circulating basophils are mature cells with a life span of about 60 h. Injection of IL-3 increases basophil generation in bone marrow, resulting in an increase in the number of circulating basophils. By contrast, mast cells in the peripheral tissue have the potential to proliferate in response to IL-3 and IL-9 *in situ*. Thus, intestinal helminth infection induces intestinal mast cell hyperplasia in an IL-3-dependent manner. A study of the development of mouse eosinophils, basophils, and mast cells has identified that granulocyte/monocyte progenitor (GMP) is a common progenitor of basophils, mast cells, and eosinophils [11]. Both basophil/mast cell precursors (BMCP) and eosinophil precursor (EoP) arise from GMP. Development from GMP to EoP and BMCP, and from BMCP to basophil precursors (BaP) and mast cell precursors (MCP), are regulated by the level and order of expression of transcription factors, C/EBP α , and, GATA-2 [11]. However, in the case of development of human granulocytes, common myeloid progenitor (CMP) develops into GMP and EoP, which then develop into basophils/mast cells and eosinophils, respectively [11].

The major function of basophils as effector cells

Although basophils and mast cells are regarded as important effector cells in allergic response by their potential to promptly produce chemical mediators and cytokines after Fc ϵ R1 cross-linkage [1,2,3*,4*,5], recent studies suggest that basophils also induce IgE-mediated chronic allergic inflammation and IgG1-mediated systemic anaphylactic shock [4*,12,13*]. Karasuyama and colleagues demonstrated that a single injection of multivalent antigen in the ear of mice passively sensitized with antigen-specific IgE, elicits immediate-phase, late-phase, and delayed onset of ear swelling characterized by infiltration with basophils and eosinophils (chronic allergic inflammation) [12]. They showed that mast cell-deficient mice only developed chronic allergic inflammation, while basophil-depleted mice failed to develop it, suggesting that basophils are responsible for inducing chronic allergic inflammation. Very recently, a study by Voehringer and colleagues has confirmed this observation by using basophil-deficient mice (*Mcp8Cre*), which constitutively lack more than 90% of basophils [14**]. Furthermore, they demonstrated that reconstitution of *Mcp8Cre* mice with bone marrow basophils restored this IgE-mediated chronic allergic inflammation response [14**]. These results strongly indicate that basophils are required and sufficient to induce IgE-mediated chronic allergic inflammation by recruiting eosinophils [12,14**].

It is well documented that mast cells and IgE are crucially involved in the development of systemic anaphylaxis. However, interestingly, mice deficient for mast cells, IgE, or Fc ϵ R1 α chain still develop systemic anaphylaxis,

indicating involvement of an alternative pathway. Fc γ R-deficient mice that lack the expression of Fc ϵ R1 and stimulatory Fc γ R do not develop systemic anaphylaxis, suggesting that IgG also plays a crucial role in induction of systemic anaphylaxis. Karasuyama and colleagues demonstrated that basophils and IgG1 contribute to certain type of mast cell-independent systemic anaphylaxis [13*]. They demonstrated that basophils released a large amount of platelet-activating factor (PAF) when stimulated with allergen-IgG1 immune complexes. Based on this result, they speculated that basophils induce systemic anaphylaxis through the release of PAF that is 30,000 times more potent than histamine [13*]. However, basophil-ablated *Mcp8Cre* mice are shown to normally develop IgE or IgG1-dependent systemic anaphylaxis, suggesting the possibility that basophils play a minor role in an induction of systemic anaphylaxis [14**]. In a previous study, Finkelman and colleagues reported that macrophages play a major role in IgG-mediated systemic anaphylaxis through the release of PAF [15]. These results suggest that both basophils and macrophage contribute to IgG1-mediated systemic anaphylaxis. However, further research is needed to determine which cell contributes most strongly to this IgG-mediated anaphylaxis.

Th subset

Naïve CD4⁺ T cells develop into Th1, Th2, Th17, and Treg cells, when they are given antigenic signals, co-stimulatory signals, and appropriate cytokine signals by APC and accessory cells [16]. IFN- γ , IL-12, and T-bet control development of Th1 cells, which are highly effective in clearance of intracellular pathogens by the production of IFN- γ [16,17]. IL-4 and GATA-3 control development of Th2 cells [16,18], which produce IL-4, IL-5, IL-6, IL-9, and IL-13. These Th2 cytokines are important for the development of allergic inflammation and clearance of helminth infections via the induction of IgE production, activation of mast cells, basophils, and eosinophils. Th17 cell subset is important for the development of autoimmune diseases and for the clearance of extracellular pathogens and fungi by producing IL-17 [19]. Differentiation of Th17 cells is induced by TGF β and IL-6 in the mouse and by TGF β and IL-6/IL-21 in the human. Treg cells are induced by TGF β and are essential for immune tolerance and regulation of allergy and autoimmunity [20].

It is well recognized that DCs play a central role in initiation of activation and differentiation of Th subsets. DCs sense microbes through TLRs and mature to express co-stimulatory molecules CD80/86 and to produce the cytokines that provide the appropriate instructive signal for the development of Th1, Th17, and Treg cells [19–21]. Antigen-pulsed DC also induce the development of Th2 cells under the influence of IL-4 *in vitro* [16]. Furthermore, several reports indicate the presence of

other pathways for the differentiation of naïve CD4⁺ T cells into Th2 cells [22–26]. DCs cannot produce IL-4, however DCs have the potential to induce Th2 cells via expression of the Notch ligand Jagged 1 and Jagged2 [22]. DCs also induce Th2 cells by expressing OX-40L after being stimulated with thymic stromal lymphopoietin (TSLP) [23]. Aluminum adjuvant induces Th2 cell differentiation, although the exact mechanism still remains uncertain [24]. In addition, M2 macrophages (also known as alternatively activated macrophages), as well as eosinophils and mast cells are also important for the development of Th2 cells [25,26]. Thus, it is important to determine which cell types help DCs to induce the development of Th2 cells.

Th2 development: basophils as accessory cells that produce early IL-4

Min *et al.* reported that naïve CD4⁺ T cells stimulated with peptide-pulsed DCs could develop into Th2 cells when co-cultured with basophils from wild type mice but not from IL-4-deficient mice [27^{**}]. As DCs and basophils are added to the same culture, it was initially interpreted that DC deliver antigenic-specific signal, and basophils provide IL-4 for the development of Th2 cells. In *in vivo* studies, mice deficient in interferon-regulatory factor 2 (IRF2) or Lyn have increased numbers of basophils and exhibit spontaneous Th2 differentiation under steady state conditions [28,29]. However, introduction of mutation in the gene encoding c-Kit inhibits this spontaneous Th2 differentiation by reducing the number of basophils [28]. Thus, basophils might be required for the development of naïve CD4⁺ T cells into Th2 cells *in vivo*. Medzhitov and colleagues showed that basophils are important in initiation of the development of Th2 cells in response to the protease allergen, papain [30^{**},31]. At day 3 after subcutaneous papain injection, basophils enter and transiently reside in the T cell zones of the draining lymph nodes, where basophils are stimulated to produce IL-4 and/or TSLP, which promote Th2 differentiation *in vivo*. Basophils are also necessary for Th2 differentiation in the mice infected with *T. muris* [32]. Depletion of basophils with antibody against FcεR1 diminished the development of Th2 cells in both models of Th2 cell differentiation, suggesting that basophils are involved in Th2 cell differentiation by production of ‘early IL-4’ [31,32]. Furthermore, basophil production of IL-4 and IL-6 promotes the development of IL-10-producing CD8⁺ T cells *in vivo* [33], suggesting that basophils play important roles for the functional differentiation of CD4⁺ T cells and CD8⁺ T cells.

Induction of Th2 cells by basophils pulsed with Ag/IgE complex

Basophils promptly produce IL-4 and IL-13 when they are stimulated with Ag/IgE complex or with IL-18 and/or IL-33 in the presence of IL-3 [5,8,9^{*},10]. Thus, if basophils express MHC class II and CD80/86, we could

speculate that basophils also have the potential to induce the development of Th2 cells. Three groups independently demonstrated that basophils constitutively express MHC class II, as well as co-stimulatory molecules such as CD40, CD80, and CD86 [31,32,34]. These groups further demonstrated that basophils are potent APCs [31,32,34]. We have reported that basophils have the capacity to induce Th2 differentiation both *in vitro* and *in vivo* [34].

We prepared splenic basophils from mice inoculated with *S. venezuelensis*, as helminth infection markedly induces an increase in the number of basophils in the spleen and liver [35]. Basophils from infected mice have the capacity to strongly produce IL-4, IL-6, and IL-13 in medium alone, even without IL-3 [34]. Furthermore, they express MHC class II and CD80/86 and induce the development of OVA-specific naïve CD4⁺ T cells into Th2 cells *in vitro* in the presence of OVA peptide, IL-2 and IL-3 without IL-4 (neutral culture condition) [34]. Thus, we initially regarded only those basophils derived from infected mice to be potent Th2 cell-inducing APC. However, we quickly learned that splenic basophils derived from naïve mice also produce IL-4, IL-6, and IL-13 in IL-3-containing medium [34]. Compared to the amount of cytokines produced by basophils from infected animals, the amount of cytokines produced by basophils from naïve mice is relatively low. Furthermore, they need the presence of IL-3 in the culture medium to produce these cytokine [7,34]. Nevertheless, splenic basophils from naïve mice express comparable levels of MHC class II and CD80/86 and have the capacity to induce the development of Th2 cells *in vitro* under neutral conditions. Thus, both types of basophils are potent APC that strongly induce Th2 cells *in vitro*.

We next tested whether bone marrow basophils also have the potential to induce the development of Th2 cells. We found that they express MHC class II, CD80, CD86, and CD62L, and take-up allergen such as OVA, and process them into small peptides [34]. Since they express FcεR1 abundantly, we speculated that they can take up a low dose of Ag/IgE complex, present Ag/MHC class II, and produce IL-4. Thus, we examined whether basophils become very potent Th2 cell-inducing APC, when Ag is provided as Ag/IgE complexes [34].

We prepared OVA-pulsed basophils by culturing basophils with DNP-OVA and anti-DNP IgE complexes. Then, we intravenously (iv) administered 0.25 or 0.5 million OVA-pulsed basophils to naïve mice. This treatment strongly induced OVA-specific Th2 cells in the spleen of naïve mice. By contrast, iv administration of OVA-pulsed DCs or mast cells failed to induce Th2 cells, although OVA-pulsed DCs induced IFN-γ producing Th1 cells. Thus, basophils are very potent Th2 cell-inducing APC even *in vivo*. We next tested whether

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endogenous basophils are crucially involved in the development of Th2 cells *in vivo* by injecting DNP-OVA/anti-DNP IgE complexes into naïve mice. We found that a single iv administration of low dose DNP-OVA/anti-DNP IgE complexes into naïve mice rapidly and preferentially induced OVA-specific Th2 cells. This induction of OVA-specific Th2 by injection of Ag/IgE complex is strongly inhibited by depletion of basophils through injection of anti-FcεR1 antibody [34]. Furthermore, IL-3 treatment renders mice highly susceptible to Th2 cell-inducing action of IgE complex by increasing the number of basophils [34]. Taken together, these results strongly indicate that basophils are also potent Th2 cell-inducing APC *in vivo*.

Atopic individuals are characterized by increased numbers of basophils at sites of allergic inflammation [36–38]. Human mature basophils lack, or only weakly express, HLA-DR but have the potential to display increased HLA-DR expression when stimulated with IL-3 [34]. As levels of IL-3 and other factors may be increased at sites of allergic inflammation, we speculated that accumulated basophils might increase their HLA-DR expression and, thus, play a role as APC in peripheral tissues and augment Th2/IgE response.

To test our hypothesis, we injected Ag/IgE complexes into naïve mice, and, indeed, showed that this treatment efficiently induced Ag-specific Th2 cells *in vivo* [34]. Since naïve mice do not possess Ag-specific IgE, we speculate that this Ag/IgE complex-dependent induction of Ag-specific Th2 cell via basophils mainly account for the mechanism how repetitive exposure to allergen augment allergic inflammation. Once atopic individuals start to produce antigen-specific IgE, their concentration of Ag/Ag-specific IgE complexes may steadily increase. Subsequently, these complexes might be efficiently taken up by basophils via the FcεR1, resulting in these basophils becoming potent Th2 cell-inducing APC, which induce progressive allergic inflammation by enhancing Th2/IgE responses in these individuals. Furthermore, Ag/IgE stimulation upregulates the expression of FcεR1 on basophils [39,40]. Indeed, there is tight correlation between FcεR1 expression on basophils, and IgE levels in human peripheral blood [41], suggesting a positive feedback mechanism for IgE-mediated immediate type hypersensitivity. Furthermore, Denzel *et al.* reported that basophils could bind large amounts of antigens via FcεR1-bound IgE and produce IL-4 and IL-6, cytokines that are known to be required for antibody production in the spleen [42]. These basophils also activate Th2 type T cells, which help B cell proliferation and IgG1 production. Depletion of basophils decreased production of IgG1 [42]. Thus, Ag/IgE complexes and basophils play a crucial role in progression of allergic disease, and, therefore, antibody therapy against IgE or basophils might be

an effective therapeutic means of treating persistent allergic disease.

Basophils as Th2 cell-inducing APC in allergy and helminth-infected hosts

Two groups revealed that basophils induce Th2 cells in the absence of DCs *in vivo* [31,32]. These studies suggest that basophils are involved in allergen-induced or helminth-induced Th2 responses by functioning as Th2 cell-inducing APC [31,32]. Artis and colleagues, by using MHC II^{CD11c} transgenic mice, where MHC class II expression is restricted to CD11c⁺ DCs, demonstrated that these mice, when inoculated with *T. muris*, fail to develop Th2 responses or expel helminths [32]. They simultaneously demonstrated that this infection induced the development of Th1 cells, suggesting that CD11c⁺ cells are required for the generation of Th1 cells. Then, they demonstrated that basophils are dominant Th2 cell-inducing APCs that express IL-4 and MHC class II. They proved this by the evidence that depletion of basophils *in vivo* reduced protective Th2 cell response to *T. muris* in wild type mice [32]. However, Min and colleagues have demonstrated that basophil depletion in *N. brasiliensis*-infected mice does not affect the development of Th2 cells, suggesting that basophils may not be essential for Th2 differentiation in mice infected with the murine hookworm, *N. brasiliensis* [43]. Nevertheless, this infection induces recruitment of basophils into the draining lymph nodes with similar kinetics as those in other models, suggesting that basophils are not required for protection against primary *N. brasiliensis* infection. However, interestingly, basophils are essential for protection against secondary *N. brasiliensis* infection, even in the absence of mast cells and CD4⁺ T cells, which are both required for efficient worm expulsion during primary infection with *N. brasiliensis* [44]. A recent study by Voehringer and colleagues has demonstrated that *Mcpt8Cre* mice with constitutive and selective depletion of basophils, normally develop Th2 cells, a IgG1/IgE response, and eosinophilia upon infection with *N. brasiliensis* [14^{••}]. However, they further demonstrated that basophils are essential for protection against secondary *N. brasiliensis* infection [14^{••},44]. These observations suggest a complex role for basophils in host defense against parasites, and also underline the complexity of regulation of acquired Th2 immunity by innate cells.

Medzhitov and colleagues also demonstrated that skin DCs are not required for mounting Th2 responses to papain. They previously reported that, like injection of the soluble antigens of *S. mansoni* eggs, papain injections rapidly induce recruitment of basophils to the lymph node [30^{••},31]. As this treatment simultaneously induces recruitment of DC, they initially considered that basophils function as accessory cells, and DC present Ag in the presence of basophil-derived IL-4. However, they

have demonstrated that skin DC are not required for the development of Th2 cells in draining lymph nodes [31]. They subcutaneously injected papain into the ear, where skin DCs capture antigens and present Ag-derived peptides to naïve T cells at draining lymph nodes. If skin DCs capture antigens and present this in the lymph node, rapid removal of these Ag-pulsed DCs by prompt excision of the injection site should inhibit Th2 response. However, this treatment failed to inhibit development of Th2 cells, suggesting that Ag captured by skin DC is not required for induction of papain-specific Th2 development. They demonstrated that soluble papain can directly enter lymph nodes from the injection site. Furthermore, selective depletion of CD11c⁺ DCs did not inhibit Th2 development to papain, suggesting the presence of Th2-inducing APC other than DCs in the lymph nodes. Medzhitov and colleagues, as well as Artis and colleagues used CD11c-restricted diphtheria toxin receptor mice, in which CD11c-expressing DC are efficiently depleted upon delivery of diphtheria toxin [31,32]. They demonstrated that DC depletion only inhibited the development of Th1 cells without affecting the development of Th2 response. These results strongly indicate that other types of APC might be required for Th2 cytokine-dependent immune responses. Subsequently, they identified basophils as another type of Th2 cell-inducing APC, as this papain-induced Th2 cell differentiation could be blocked by the depletion of basophils by means of anti-FcεR1 antibody MAR-1 [31]. They have clearly shown that basophils can take up, process, and present soluble antigens. Furthermore, they demonstrated that basophils form immune synapses with T cells within 60 min of co-culture [31]. Finally, they demonstrated that adoptive transfer of OVA-pulsed basophils induced Th2 response in MHC class II-deficient mice.

The above-mentioned studies showed that basophils are potent APCs that preferentially induce Th2 cells both *in vitro* and *in vivo*. Mice deficient in the Src family tyrosine kinase, Lyn, have increased numbers of basophils, exhibit spontaneous Th2 differentiation, and produce IgE autoantibodies against various autoantigens, including dsDNA, that cause lupus-like nephritis in Lyn^{-/-} mice [29]. Thus, basophils regulate not only allergic diseases and anti-helminth immunity but also autoimmune diseases.

Following studies

However, recent studies questioned the role of basophils as Th2 cell-inducing APCs. Tang *et al.* suggested that DCs and basophils cooperatively induce a Th2 cell response against papain [45]. Phytian-Adams *et al.* reported that depletion of CD11c⁺ DCs during the priming stage of Th2 response against the helminth *Schistosoma mansoni* severely impaired Th2 induction, while basophil depletion using MAR-1 antibody had no mea-

surable effect, underlining the important role of DCs in the development of Th2 response against helminth infection [46]. Furthermore, Hammad *et al.* demonstrated that Th2 responses against house dust mite are initiated by FcεR1-expressing inflammatory DCs, which is amplified by basophils [47]. Voehringer and colleagues examined basophil function using newly developed basophil-ablated mice. In this model they could not find the evidence that basophils are required for Th2 cell differentiation *in vivo* [14^{••}]. They speculated that antibody-mediated depletion of basophils with MAR-1 (anti-FcεR1) may simultaneously deplete FcεR1 expressing inflammatory DCs [47]. They also suggested bystander effects of antibody-treatment, including activation of mast cells and macrophages, as well as the formation of immune complexes.

Conclusion

The data on the role of basophils as APCs are confusing but the issue is still interesting. As we reported previously [34], basophils pulsed with Ag/IgE complexes have potent Th2 cell-inducing APC activity both *in vitro* and *in vivo*. Basophils take-up intact OVA and present OVA-peptide in conjunction with MHC class II to OVA-peptide specific naïve CD4⁺ T cells. Furthermore, when injected into naïve animal, these Ag-pulsed basophils also exhibited Th2 cell-inducing APC activity *in vivo*. However, their role as initiating cells of primary Th2 response might be minor. I suspect their important role for secondary Th2 response. Nevertheless, since basophils often show strong protective effect against helminth infection, they are still very interesting cells. To fully understand the role of basophils in Th2-mediated anti-helminth immunity, we definitely need further studies of these cells *in vivo*.

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