

Basophils as antigen presenting cells

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Recent reports demonstrate that basophils act as antigen presenting cells to drive Th2 and IgE responses against protease and protein allergens and helminth parasites. Through MHC class II-dependent cognate interactions with CD4⁺ T cells in the context of co-stimulatory molecules, and through secretion of IL-4, IL-13 and thymic stromal lymphopoietin, basophils drive antigen-specific Th2 responses. These results have uncovered previously unknown functions of basophils, and should aid in designing novel therapeutic strategies for asthma and allergic conditions.

The enigma of Th2 differentiation

Antigen presenting cells (APCs) play a critical role in the uptake and processing of antigenic peptides that are then presented via MHC molecules to T cells. Three major APC types have been identified: dendritic cells (DCs), macrophages, and memory B cells. Among these, DC are considered to be professional APCs because of their ability to prime naïve CD4⁺ T cells. At present, the nature of the signals that mediate Th1, Th17, and T regulatory (Treg) cell responses are fairly well understood [1]. Th1 cells are critical for cellular immune responses against intracellular pathogens, and are differentiated in the presence of IL-12 secreted by DCs. Th17 cells mediate immune responses against extracellular pathogens and fungi. In mice Th17 cells can be differentiated in the presence of TGFβ and IL-6, whereas in humans TGFβ and IL-21 are required. Tregs are involved in maintaining immune tolerance and can be induced in the periphery under the influence of TGFβ.

Th2 cells that express the transcription factor GATA3 and secrete IL-4, IL-5, IL-9, and IL-13, are important for clearing multicellular parasites such as helminths. They are also involved in the pathogenesis of asthma and allergic conditions. Although conditioning of DCs under specific conditions can drive Th2 responses *in vitro* [2], the nature of APCs involved in mounting Th2 responses under physiological conditions is not understood clearly. Indeed, the role of DCs as the initiators of Th2 responses has been questioned for a number of reasons. First, unlike viruses and bacteria, helminths are too large to be phagocytosed directly by DCs and hence the source of antigens for APCs are either those shed or secreted from parasites. In addition, DCs undergo only limited activation upon encounter with allergens and helminths and hence cannot strongly prime Th2 responses from naïve cells. Secondly,

IL-4 is a critical cytokine for the differentiation of Th2 cells, but is not secreted by DCs. Third, Th2 responses are characterized by the recruitment and activation of basophils, eosinophils and mast cells, and secretion of IL-4 by these cells [3]. Fourth, basophils are recruited to lymph nodes in response to protease allergens, and due to their ability to secrete early IL-4 and thymic stromal lymphopoietin (TSLP), basophils promote Th2 differentiation [4]. Fifth, basophils are activated by secreted antigens of the helminth *Nippostrongylus brasiliensis* in an antigen-specific manner and basophil-depleted mice show impaired expulsion of these worms [5]. In addition, *in vitro* studies have demonstrated that only basophils can produce IL-4 and IL-13 in response to a combination of IL-3, and IL-18 or IL-33 [6,7]. Collectively, these lines of evidence support a critical role for basophils in Th2 differentiation.

Dendritic cells are insufficient to prime Th2 responses *in vivo*

Recently three independent groups have tried to identify the nature of APCs implicated in Th2 responses [8–10]. Artis and colleagues first studied whether CD11c⁺ DCs are required to induce Th2 responses *in vivo* to the murine nematode *Trichuris muris* [9]. By using MHC II^{CD11c} transgenic mice, where MHC II expression is restricted to CD11c⁺ DCs, they demonstrated that DC-restricted MHC II expression was insufficient for inducing Th2 cytokines, priming Th2 responses, and to clear parasites from the intestinal tract. In addition, deficient expression and secretion of the intestinal goblet cell-specific, and Th2 cytokine-dependent immune-effector molecule RELMβ in MHC II^{CD11c} transgenic mice indicated that CD11c⁺ DCs are not sufficient to mount protective Th2 responses to gastrointestinal helminth parasites. A study by Medzhitov and colleagues supported these results by demonstrating that skin DCs are not required for mounting Th2 responses to the protease allergen papain [8]. Skin DCs capture antigens in the periphery and present antigen-derived peptides to naïve T cells at draining lymph nodes. If skin DCs are implicated in the induction of Th2 responses to papain, removal of this antigen from the injection site should inhibit Th2 responses. On the contrary, Medzhitov and colleagues found that Th2 responses to papain were induced despite excision of the injection site, suggesting that antigen capture by skin DCs is not required for inducing papain-specific Th2 differentiation, and that soluble papain can directly enter lymph nodes and access APCs [8]. Further, restricting the MHC II expression to DCs by using MHCII^{CD11c} mice, prevented the Th2 responses to papain, similar to the *T. muris* experiments

[9]. Interestingly, selective depletion of CD11c⁺ DCs did not affect Th2 responses to papain or *T. muris* although the ability of mice to mount Th1 responses was impaired severely [8,9]. These results thus indicate that, while DCs are critical cells for Th1 responses, they are dispensable for Th2 responses *in vivo*, and hence another APC might be required for Th2 cytokine-dependent immunity. Multiple lines of evidence support the contention that basophils act as professional APCs for mounting Th2 responses against protease and protein allergens, as well as helminths: i) basophils are the major cells recruited to lymph nodes upon injection of papain, *T. muris*, and eggs of the helminth parasite *Schistosoma mansoni*, and basophils express both *Il4* mRNA and MHC II [4,8,9]; ii) impaired Th2 responses to papain and *T. muris* in mice where MHC II expression is restricted to DCs [8,9]; and iii) CD11c⁺ DCs are capable of giving signals for protective Th2 responses to *T. muris*, only if IFN γ , a Th1-inducing cytokine is neutralized [9].

Basophils display classical features of antigen presenting cells

Basophils represent less than 1% of circulating leukocytes, and have been linked to immune responses in allergic conditions and parasite infections. Basophils display classical features of APCs, such as expression of MHC class I and II, co-stimulatory molecules CD40, CD80 and CD86, and adhesion molecule CD54 [8–11] (Box 1). However, the expression of MHC was found to be lower compared to that of other APCs, including DCs and B cells. In addition, bone marrow-derived basophils express the lymph node-homing marker CD62L, suggesting their potential to migrate to lymphoid tissues to initiate T cell responses *in vivo*. Interestingly, a fraction of basophils isolated from human cord blood was also shown to express HLA-DR [10], thus confirming that MHC class II expression of basophils is not unique to mice.

Basophils induce Th2 differentiation *in vitro*

Basophils induce Th2 differentiation *in vitro* both under neutral conditions and antigen-specific conditions, with no role for any DC subsets in this function. This *in vitro* Th2 differentiation was enhanced strongly when basophils were activated by Th2 antigens, and was correlated with enhanced secretion of Th2-differentiating basophil cytokines IL-4 and IL-13 [4,8–10]. Further, the priming and differentiation of Th2 by basophils was dependent on MHC Class II antigen presentation and IL-4 secretion, indicating that basophils act as classical APCs by processing and presenting antigens (Figure 1). In contrast to DCs, however, basophils are found to endocytose only soluble antigens but not particulate antigens [8].

Basophils act as antigen presenting cells *in vivo* to prime Th2 responses

S. mansoni egg-elicited basophils influence CD4⁺ T cell proliferation *in vivo* upon adoptive transfer and subsequent antigen challenge [9]. Further, depletion of basophils using a monoclonal antibody to Fc ϵ RI, results in a decline in both *Il4* mRNA and intestinal lumen secretion of RELM β and a consequent impairment of protective

Box 1. Features of basophils allowing them to function as antigen presenting cells for Th2 immunity

- Expression of MHC class II molecules
- Expression of co-stimulatory (CD80, CD86, CD40) and adhesion (CD54) molecules
- Expression of Fc ϵ R to capture IgE-antigen complexes
- Ability to undergo activation upon encounter with Th2 antigens
- Ability to endocytose and present antigens in the context of MHC class II
- Ability to migrate to lymphoid tissues to initiate Th2 responses
- Secretion of Th2-polarizing cytokines IL-4, IL-13, and thymic stromal lymphopoietin (TSLP)

immunity to *T. muris* [9]. Similarly, adoptive transfer of antigen-loaded MHC class II-sufficient basophils into MHC class II-deficient mice (i.e. *Ciita* or I-A^b-deficient) demonstrated that basophils alone are sufficient for papain-induced Th2 responses *in vivo* [8]. Finally, adoptive transfer experiments by Nakanishi and colleagues showed that basophils pulsed with 2,4-dinitrophenyl-conjugated ovalbumin (DNP-OVA) and IgE-anti-DNP induce strong Th2 responses and produce OVA-specific IgE and IgG1 [10]. This group further demonstrated that basophils endocytose efficiently DNP-OVA-IgE-anti-DNP complexes to induce OVA-specific Th2 responses, and basophil-depleted mice fail to mount Th2 responses when injected with DNP-OVA-IgE-anti-DNP complexes (Figure 1). As previously reported, basophils also support immunological memory responses by enhancing the CD4⁺ T cell help to B cells and by their secretion of IL-4 and IL-6 [12]. Together, these data can explain how basophils initiate allergic inflammation. Indeed, a role for basophils in IgE-mediated chronic allergic inflammation has been demonstrated [13].

Are basophils the only antigen presenting cell for Th2 responses?

Recent reports compellingly demonstrate that basophils are the physiological APC for inducing Th2 responses to protease and protein allergens and helminth parasites. However, Th2 responses are highly heterogeneous, and several reports have demonstrated previously that under certain conditions, other APCs can also induce Th2 responses. The Notch ligand Jagged can trigger Th2 differentiation by DCs independent of IL-4 and STAT6 signaling [2]. By enhancing the expression of OX40 ligand on DCs, the soluble extract of *S. mansoni* eggs induces DC-mediated Th2 cells [14,15]. Aluminum adjuvants induce Th2 differentiation by activating the NALP3-inflammasome in macrophages and DCs [16]. In addition, M2 (also known as alternatively activated macrophages), eosinophils, and mast cells have the ability to act as APCs to induce Th2 differentiation [17–19]. Therefore, it is possible that basophils are not the only APC that mediate Th2 responses to all antigens. In fact, Artis and colleagues also suggest potential cooperation of DCs and basophils in efficient expansion of helminth-specific CD4⁺ T cells *in vivo* [9]. Deciphering the pathogen recognition and endocytic receptors on basophils, intracellular signaling pathways, and the collaboration of other classical APCs with basophils should help to solve this mystery. As described

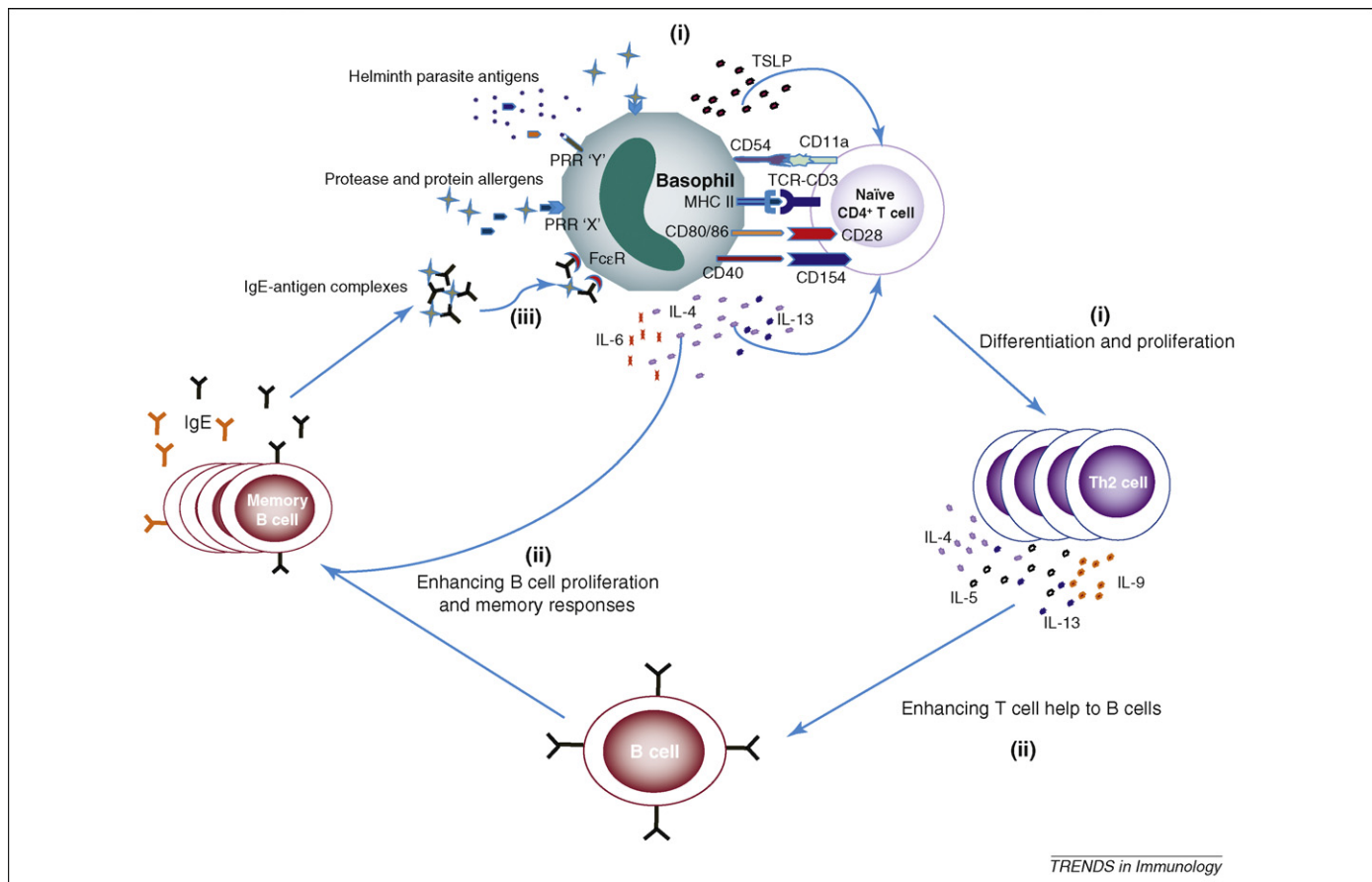


Figure 1. Basophils act as physiological antigen presenting cells to induce Th2 and IgE responses to protease and protein allergens as well as helminth parasites. (i) Through MHC class II-dependent cognate interactions with CD4⁺ T cells in the context of co-stimulatory molecules (CD80, CD86, CD40 and CD54), and through secretion of IL-4, IL-13, and thymic stromal lymphopoietin (TSLP), basophils drive antigen-specific Th2 responses. (ii) Basophils also enhance humoral memory responses by enhancing the CD4⁺ T cell help to B cells, and by secreting IL-4 and IL-6. (iii) However, pathogen recognition and endocytotic receptors (indicated as PRR 'X' and PRR 'Y') on basophils have yet to be identified, although antigen-specific IgE produced by B cells can form immune complexes with antigens. By endocytosing IgE-complexed antigens via FcεR, basophils can induce Th2 responses. Consequently, FcεR has a prominent role in memory responses, but not in inducing primary responses.

above, basophils induce Th2 responses by endocytosing IgE-complexed antigens via FcεR [10]. However, FcεR has a prominent role only in memory responses, but not in inducing primary responses. Therefore, research on the role of basophils in chronic allergic diseases and the molecular mechanisms of induction of Th2 responses should aid identification of therapeutic molecules that block this arm of the immune system.

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