

Research Highlight

The Emergence of Basophils as Antigen-Presenting Cells in Th2 Inflammatory Responses

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Basophils gain prominence in Th2 inflammatory responses with the discovery that they function as antigen-presenting cells and are sufficient to drive Th2 cell differentiation.

The Th2 inflammatory response is critical for host defense against helminthic parasites, but it is also responsible for the pathogenesis of allergic disorders, such as asthma. Tissue infiltration with eosinophils, mast cells, basophils and lymphocytes as well as mucus hypersecretion due to goblet cell hyperplasia and smooth muscle hyperresponsiveness characterize Th2 inflammation in the airway. Central to Th2 immunity are antigen-specific Th2 cells, which are specialized CD4⁺ T helper cells that secrete IL-4, IL-5 and IL-13 after antigen encounter. Upon arrival in tissue, antigen-specific Th2 cells augment the Th2 cytokine milieu and propagate Th2 inflammation by attracting more inflammatory cells (Medoff et al., 2008).

Differentiation of naïve CD4⁺ T cells into Th2 cells requires three signals: T cell receptor triggering through peptide antigen recognition in the context of MHC class II molecule, amplification of T cell receptor signaling via co-stimulatory molecules and the presence of appropriate cytokine. In the case of Th1 and Th17 cells, which develop in response to viral, bacterial and fungal pathogens, dendritic cells function as the antigen-presenting cells and provide all three signals. Dendritic cells use pattern recognition receptors, such as Toll-like receptors, to recognize pathogen-associated molecular patterns. This results in dendritic cell activation, up-regulation of MHC class II and co-stimulatory molecules and dendritic

cell release of relevant cytokines, such as IL-6, IL-23 and IL-12. For Th2 cell differentiation, however, dendritic cells are not the one-stop shop for all three required signals since dendritic cells do not produce IL-4, the cytokine necessary for Th2 cell differentiation. This observation implies that other antigen-presenting cells, in addition to dendritic cells, may contribute to Th2 cell differentiation. In fact, eosinophils, the main effector cell population infiltrating sites of allergic inflammation and helminth parasitic infections, can express MHC class II, produce IL-4 and induce proliferation and IL-4 production in naïve CD4⁺ T cells (Wang et al., 2007). In addition, mast cells, another key cellular player in Th2 inflammation, have been shown to express MHC class II after Delta-like 1/Notch signaling and subsequently gain the ability to induce CD4⁺ T cell proliferation and Th2 cell differentiation *in vitro* (Nakano et al., 2009). In three recent papers in *Nature Immunology*, Artis and colleagues (Perrigoue et al., 2009), Medzhitov and colleagues (Sokol et al., 2009) and Nakanishi and colleagues (Yoshimoto et al., 2009) present convincing evidence that basophils also function as antigen-presenting cells to promote Th2 inflammatory responses.

To function as an antigen-presenting cell, the cell must fulfill a number of criteria. Upon antigen exposure, the candidate cell must express MHC class II and co-stimulatory molecules. It must be able

to take up and process antigen and localize to draining lymph nodes, where efficient T cell–antigen-presenting cell interactions take place. Ideally, the candidate cell would also express the relevant cytokines required for T cell differentiation, although other accessory cells may provide the required cytokines. Finally, the candidate cell must be able to induce CD4⁺ T cell proliferation and differentiation in *in vitro* and *in vivo* assays. The studies by Artis and colleagues (Perrigoue et al., 2009), Medzhitov and colleagues (Sokol et al., 2009) and Nakanishi and colleagues (Yoshimoto et al., 2009) use three different models and elegantly demonstrate that basophils meet the criteria for antigen-presenting cells for Th2 cell differentiation.

Artis and colleagues studied the Th2 inflammatory response to the intestinal helminth parasite *Trichuris muris*. They showed that mice with CD11c-restricted expression of MHC class II did not produce IL-4, IL-5 and IL-13 or mucin in response to *T. muris* and were unable to clear the infection compared with wild-type mice. They observed that basophils expressed MHC class II and IL-4 and comprised 40% of the IL-4⁺ MHC class II⁺, non-B, non-T cell population after helminth infection. IL-3-activated, OVA-pulsed basophils promoted the proliferation and Th2 differentiation of OVA-specific CD4⁺ T cells *in vitro*, and these responses were abrogated with the addition of MHC class II blocking antibody. The authors then

expanded their model to *Schistosoma mansoni*, another helminth parasite. They demonstrated that the number of basophils increased substantially in the draining lymph node following infection with *S. mansoni*. The authors then isolated basophils from draining lymph nodes of mice infected with *S. mansoni* and transferred these wild-type basophils into mice that had CD11c-restricted MHC class II expression. A group of these mice were not given basophils. All mice were infected with *S. mansoni*, and CD4⁺ T cell proliferation was compared between the two groups. The authors found that *in vivo* transfer of wild-type basophils into *S. mansoni*-infected mice with CD11c-restricted MHC class II expression augmented the proliferation of CD4⁺ T cells in draining lymph nodes (Perrigoue et al., 2009). In summary, this study showed that CD11c-restricted expression of MHC class II was not sufficient to generate a Th2 inflammatory response.

Medzhitov and colleagues investigated the role of basophils in Th2 response to protease antigen papain and OVA. The authors demonstrated that bone marrow-derived basophils, but not bone marrow-derived dendritic cells, were able to induce IL-4 production in OVA-specific CD4⁺ cells in response to OVA peptide *in vitro*. Papain exposure induced basophil recruitment into the draining lymph nodes, increased basophil MHC class II expression and enhanced the ability of basophils to drive Th2 cell differentiation, whereas MHC class II blocking antibody and basophil IL-4 deficiency abrogated these responses. Basophils endocytosed FITC-labeled OVA, expressed MHC class II and co-stimulatory molecules, and were able to form immunologic synapses with OVA-specific T cells in culture. Furthermore, OVA-loaded, bone marrow-derived basophils from Bcl-2 transgenic mice, which have improved survival and express MHC class II, were able to induce Th2 cell differentiation in I-A^b-deficient and CIITA^{2/2} mice (Sokol et al., 2009). This study demonstrated that MHC expression on basophils was sufficient to drive Th2 cell differentiation.

Nakanishi and colleagues focused on the role of basophils in augmentation of Th2 responses by antigen-IgE immune complexes. They began by showing that

splenic basophils from mice infected with *Strongyloides venezuelensis*, as well as bone marrow-derived basophils from uninfected mice, expressed Th2 cytokines and MHC class II and induced IL-4 production in naïve OVA-specific CD4⁺ cells under neutral conditions *in vitro*. IL-4 deficiency abrogated the ability of basophils to induce Th2 cell differentiation. Bone marrow-derived basophils were able to induce Th2 cell differentiation in naïve OVA-specific CD4⁺ cells in response to DNP-OVA and this ability increased with the addition of anti-DNP IgE. In another approach, bone marrow-derived basophils, mast cells and splenic dendritic cells were incubated *in vitro* with DNP-OVA and anti-DNP IgE and then transferred into wild-type mice, which were challenged subsequently with intravenous OVA. Splenic CD4⁺ cells from dendritic cell recipient mice differentiated into a Th1 phenotype, those from mast cell recipients did not differentiate, while those from basophil recipient mice differentiated into a Th2 phenotype. Finally, IL-3 treatment of naïve mice resulted in expansion of basophils *in vivo* and increased the Th2 response to DNP-OVA plus anti-DNP IgE (Yoshimoto et al., 2009). This study highlights that IgE augments the ability of basophils to induce Th2 cell differentiation in naïve CD4⁺ T cells.

With basophils added to the list of cells capable of antigen presentation to naïve CD4⁺ T cells, the question arises as to what is the dominant antigen-presenting cell in Th2 inflammatory responses. There is a large body of literature on the role of dendritic cells in Th2 inflammation. For example, intratracheal transfer of antigen-pulsed myeloid dendritic cells followed by airway antigen challenges has been shown to be sufficient to induce eosinophilic airway inflammation (Lambrecht et al., 2000). Consistently, obliteration of dendritic cells using diphtheria toxin in mice expressing diphtheria toxin receptor in CD11c⁺ cells (CD11c-DTR) abrogates features of allergic airway inflammation (van Rijt et al., 2005). The studies by Artis and colleagues (Perrigoue et al., 2009) and Medzhitov and colleagues (Sokol et al., 2009) challenge the paradigm that dendritic cells are the dominant antigen-presenting cells in Th2 cell differentiation and instead identify basophils as the

necessary and sufficient antigen-presenting cell in Th2 cell differentiation in their models. Four approaches were used to highlight the dominant role of basophils.

First, the authors showed that basophil depletion through treatment with a monoclonal antibody to Fc ϵ RI significantly diminished Th2 cell differentiation (Yoshimoto et al., 2009) and Th2 immunity to *T. muris* (Perrigoue et al., 2009). The authors did not address that this monoclonal antibody also decreases the number of mast cells (Denzel et al., 2008), and may also lower Th2 cell differentiation by removing two early sources of IL-4 (Sullivan and Locksley, 2009).

The second approach used diphtheria toxin in CD11c-DTR mice and demonstrated that depletion of dendritic cells did not alter Th2 cell differentiation in response to OVA plus papain (Sokol et al., 2009) or Th2 immunity to *T. muris* (Perrigoue et al., 2009). It is noteworthy that both studies used CD11c-DTR bone marrow chimeras, in which persistence of tissue resident wild-type, and diphtheria toxin-unresponsive dendritic cells was not ruled out.

The third approach demonstrated that CD11c-restricted expression of MHC class II was not sufficient to induce Th2 cell differentiation in response to OVA plus papain (Sokol et al., 2009) or to protect against infection with *T. muris* (Perrigoue et al., 2009). However, the study by Artis and colleagues illustrated that Th1 cell differentiation was intact in CD11c-MHC II mice. In fact, neutralization of endogenous IFN- γ restored IL-4, IL-5 and IL-13 production as well as mucous hypersecretion and decreased worm burden (Perrigoue et al., 2009). This implies that MHC II expression on CD11c⁺ cells was sufficient to drive Th2 inflammation as long as the right cytokine milieu was present.

In the fourth approach, mice were immunized in the ear with papain and the ear was either left intact or removed after two hours in order to remove the source of tissue resident dendritic cells. The authors observed Th2 cell differentiation, albeit at lower levels, in the draining lymph node, despite removal of skin resident dendritic cells (Sokol et al., 2009). However, it should be noted that while this approach may have excluded tissue resident dendritic cells from antigen

presentation in the lymph node, dendritic cells in the lymphatic conduits were still intact and could have taken up soluble antigens *en route* to the lymph node.

The observation that multiple cell types implicated in Th2 inflammation, such as basophils, eosinophils, mast cells and dendritic cells, are all capable of presenting antigens to promote Th2 inflammation may reflect an evolutionary strategy for redundancy to ensure a robust Th2 response to parasitic infections. Alternatively, the different cell types may function as dominant antigen-presenting cells under different circumstances with basophils playing a dominant role during infections with helminth parasites as demonstrated by Artis and colleagues (Perrigoue et al., 2009) or in response to protease antigens as presented by Medzhitov and colleagues (Sokol et al., 2009). The idea that the basophil is the dominant antigen-presenting cell for Th2 cell differentiation is certainly intriguing,

but its validation requires further investigation.

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