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## Basophils trump dendritic cells as APCs for T<sub>H</sub>2 responses

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### Abstract

Dendritic cells are best known as antigen-presenting cells that initiate adaptive immune responses. Three new papers suggest that basophils initiate allergen- and helminth-driven CD4<sup>+</sup> T helper type 2 responses by functioning as antigen-presenting cells in draining lymph nodes.

Although the cellular and molecular mechanisms that regulate the development of T helper type 1 cell (T<sub>H</sub>1 cell), interleukin 17 (IL-17)-producing T helper cell (T<sub>H</sub>-17 cell) and regulatory T cell responses are fairly well understood, the specific cellular mediators and factors that control the initiation of T<sub>H</sub>2 responses are still highly debated. Although it is certain that dendritic cells (DCs), pattern-recognition receptors and cytokines secreted by DCs are key in the initiation and expansion of most effector and regulatory T cell classes, the relative importance of activated DCs and Toll-like receptor signaling in the development of T<sub>H</sub>2 effector responses is less clear. In this issue of *Nature Immunology*, three papers demonstrate that DCs are not required for the generation of CD4<sup>+</sup> T<sub>H</sub>2 responses to protease allergens<sup>1</sup>, helminthic parasites<sup>2</sup> or antigen-immunoglobulin E (IgE) complexes *in vivo*<sup>3</sup>. Instead, all three groups identify the major histocompatibility complex (MHC) class II-positive IL-4-producing basophil as the ‘professional’ antigen-presenting cell (APC) that is both necessary and sufficient for the generation of type 2 immunity.

Studies have suggested that DCs adopt a fairly limited activation profile when exposed to T<sub>H</sub>2-inducing allergens and helminths<sup>4</sup>. They also fail to produce IL-4, the key driver of CD4<sup>+</sup> T<sub>H</sub>2 cell responses. Therefore, attention has focused on identifying the accessory cells that provide the early innate source of IL-4 and soluble mediators that ‘instruct’ DC-mediated T<sub>H</sub>2 differentiation. Proposed sources of IL-4 have included eosinophils, mast cells, basophils and natural killer T cells, as well as autocrine IL-4 from CD4<sup>+</sup> T cells<sup>5–8</sup>. Additional cytokine cofactors have also been identified, including IL-21, IL-25, IL-33 and thymic stromal lymphopoietin, which invariably augment development of T<sub>H</sub>2 responses by modulating the activation status of DCs and other APCs<sup>9–12</sup>. The long-standing view of T<sub>H</sub>2 differentiation has revolved around this basic theory, which suggests CD4<sup>+</sup> T<sub>H</sub>2 cell development is driven by DCs that present antigen in the context of MHC class II and by extrinsic cellular and secreted factors that modify DC maturation and provide an early source of IL-4 (refs. <sup>13,14</sup>).

Sokol and colleagues investigate the mechanisms that regulate the development of T<sub>H</sub>2 responses after exposure to papain, a cysteine protease hydrolase enzyme from papaya that breaks down complex proteins and thus mimics the activity of proteases secreted by many T<sub>H</sub>2-promoting helminth parasites. Although basophils are found mainly in the blood and peripheral tissues, they are rapidly recruited to the lymph nodes during a primary response to papain and in response to the soluble antigens of *Schistosoma mansoni* eggs<sup>15</sup>. Once in the

lymph nodes, the basophils secrete IL-4 and thymic stromal lymphopoietin, and depletion studies suggest that basophils are critically involved in the generation of antigen-specific T<sub>H</sub>2 responses. The conclusion reached was that basophils function as accessory cells for DC-mediated T<sub>H</sub>2 differentiation because DCs are also rapidly recruited to the lymph nodes. Nevertheless, the specific identify of the APC population was unclear in those studies<sup>15</sup>. In the manuscript presented here, Sokol and colleagues show that, unexpectedly, DCs are in fact not required for the development of papain-induced T<sub>H</sub>2 responses<sup>1</sup>. Although papain-primed DCs initiate the development of T<sub>H</sub>2 responses *in vivo*, they are not able to induce CD4<sup>+</sup> T<sub>H</sub>2 cells *in vitro* unless basophils are included in the culture. Surprisingly, these authors discover that DCs are not even necessary, as basophils alone support the robust proliferation of naive T cells. Thus, unlike the *in vitro* generation of T<sub>H</sub>1 and T<sub>H</sub>-17 responses, for which DCs, antigen and Toll-like receptor signals are sufficient, T<sub>H</sub>2 responses exploit a distinct basophil-dependent but DC-independent mechanism. These findings are unexpected, as basophils have been thought to be MHC class II negative; however, these authors show very convincingly that some activated basophils express MHC class II. Basophils also have the molecular 'machinery' required to function as APCs, as shown by *in vitro* MHC class II-blocking studies. The T<sub>H</sub>2 cells generated are also papain specific. These findings collectively provide evidence that basophils can function as professional APCs, at least for the generation of T<sub>H</sub>2 responses *in vitro*.

During the *in vivo* generation of T<sub>H</sub>1 or T<sub>H</sub>-17 responses, DCs encounter pathogens in peripheral tissues, where they sample foreign antigens, become activated and then migrate to the lymph nodes, where they present antigen in the context of MHC class II to naive T cells (Fig. 1). Sokol *et al.* seek to determine whether basophils use a similar mechanism to initiate T<sub>H</sub>2 responses or require DCs to escort papain into the draining lymph node, where antigen encounter occurs<sup>1</sup>. To answer these questions, they design a clever set of experiments in which they inject papain into the ear pinna of mice and then excise the ear at either 2 h or 24 h after injection. If DCs are needed to capture and deliver antigen to lymph nodes, rapid excision of the injection site would ablate the development of the T<sub>H</sub>2 response in the draining lymph node. Interestingly, they find no difference in T<sub>H</sub>2 development at 2 h and 24 h, which suggests that migratory DCs are not involved and that soluble proteins such as papain are being delivered directly to the lymph node. They also show that basophils can endocytose, process and present soluble antigens, but unlike DCs, they are not good at processing particulate antigens. They hypothesize that because most T<sub>H</sub>2-inducing antigens from helminth parasites are excretory or secretory proteins, this mechanism would be ideally suited for the generation of T<sub>H</sub>2 responses to large extracellular eukaryotic pathogens. In support of that hypothesis, Perrigoue *et al.* show that MHC class II-positive DCs are not required for the generation of protective CD4<sup>+</sup> T<sub>H</sub>2 cell-dependent immunity to the gastrointestinal nematode parasite *Trichuris muris*<sup>2</sup>. Similar to the studies by Sokol and colleagues<sup>1</sup>, the results of Perrigoue *et al.* show that IL-4-producing basophils are MHC class II positive and can promote the MHC class II-dependent differentiation of antigen-specific CD4<sup>+</sup> T<sub>H</sub>2 cells *in vitro*<sup>2</sup>. More importantly, depletion of basophils *in vivo* with a monoclonal antibody (Mar-1) specific for the receptor FcεRI considerably impairs immunity to *T. muris*, which suggests that basophils facilitate the development of protective T<sub>H</sub>2 immunity, thus extending the findings of Sokol *et al.* regarding papain to a complex T<sub>H</sub>2-promoting pathogen.

Basophils isolated from the spleens of mice infected with the intestinal nematode *Strongyloides venezuelensis* are also MHC class II positive, as shown by Yoshimoto *et al.*<sup>3</sup>. Splenic basophils from infected mice also secrete IL-4 and, in agreement with the other two studies<sup>1,2</sup>, these cells are able to induce the development of antigen-specific T<sub>H</sub>2 cells *in vitro* in the absence of DCs<sup>3</sup>. Yoshimoto *et al.*<sup>3</sup> show that IL-4-deficient basophils are not functional, demonstrating that the production of IL-4 by MHC class II-positive basophils is critical for T<sub>H</sub>2 differentiation. Interestingly, basophils from naive mice have the same T<sub>H</sub>2-inducing ability, so IgE-primed

basophils do not seem to be important. Nevertheless, enhanced T<sub>H</sub>2 responses result when antigen-IgE complexes are included in the culture, which suggests that antigen-specific IgE augments the development of antigen-specific T<sub>H</sub>2 responses, perhaps by facilitating antigen uptake. Yoshimoto *et al.*<sup>3</sup> also discover that basophils express the lymph node-homing receptor CD62L, which indicates that basophils have the necessary 'machinery' to enter secondary lymphoid tissues where T<sub>H</sub>2 responses are initiated. Perhaps most importantly, however, they determine that IL-3 can induce HLA-DR expression on a subset of human basophils. Thus, these important findings may not be restricted to the mouse.

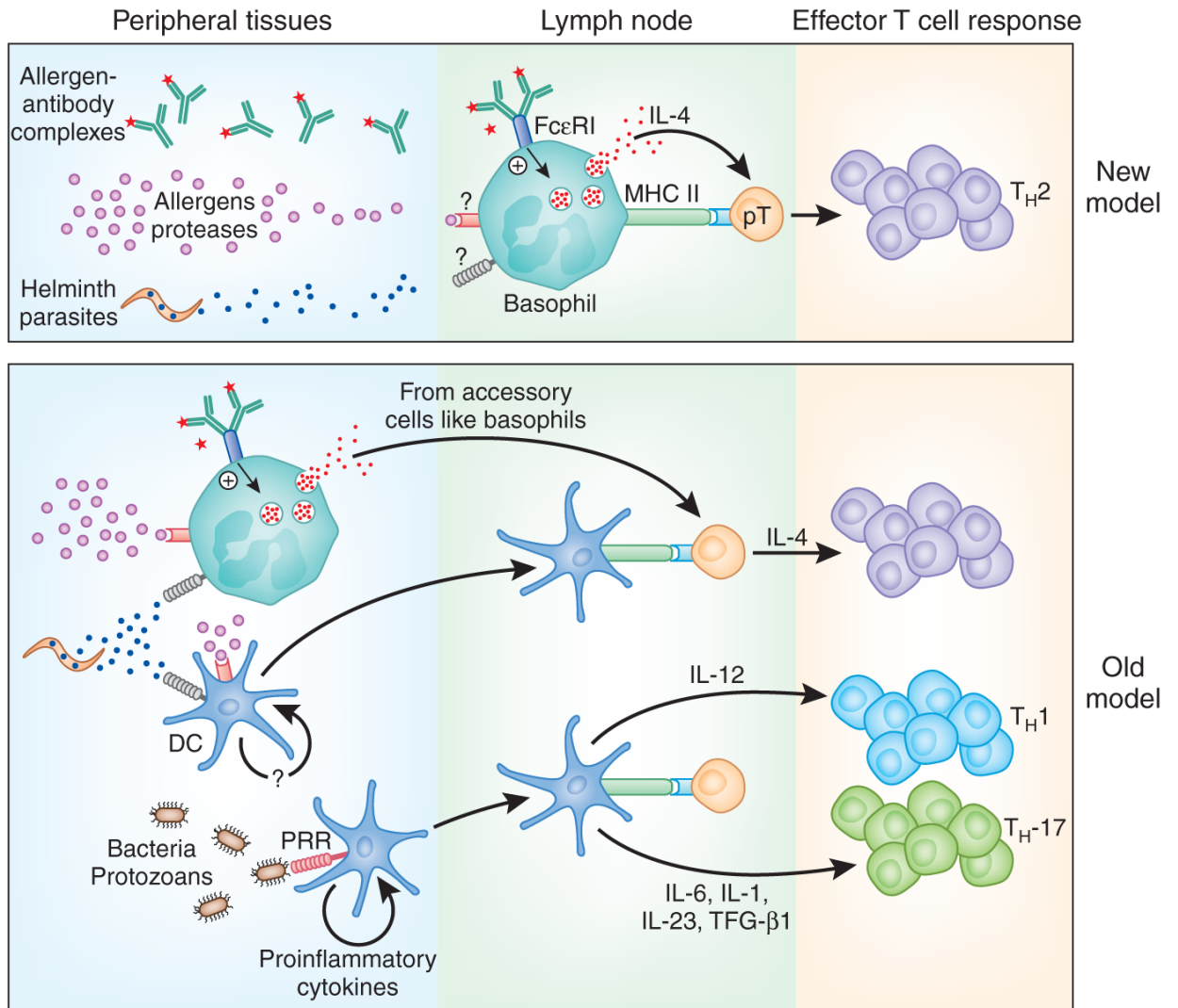
Although all three groups show that MHC class II-positive basophils can initiate T<sub>H</sub>2 differentiation *in vitro* in the absence of other professional APCs, it is important to confirm this mechanism *in vivo*. To do this, all three groups use similar and complimentary approaches to rule out the possibility that DCs are involved. Sokol *et al.*<sup>1</sup> and Perrigoue *et al.*<sup>2</sup> both use the CD11c-diphtheria toxin receptor mouse model in which delivery of diphtheria toxin effectively depletes the mice of all CD11c-expressing cells<sup>16</sup>. Sokol *et al.* show that although depletion of DCs blocks T<sub>H</sub>1 differentiation, it has no effect on papain induced T<sub>H</sub>2 responses<sup>1</sup>. Similarly, Perrigoue *et al.* find that these mice do not have diminished development of protective T<sub>H</sub>2 immunity after *T. muris* infection<sup>2</sup>. Both groups also take the opposite approach by restricting MHC class II expression to DCs<sup>17</sup>. Here again, although MHC class II-positive DCs are adequate for the development of T<sub>H</sub>1 responses, they are not sufficient for the development of T<sub>H</sub>2 responses *in vivo*. Although IL-4 producing basophils are recruited to the lymph nodes in these studies, expression of MHC class II on basophils seems to be critical for the development of the T<sub>H</sub>2 response. Notably, however, when the mice in which MHC class II expression is restricted to DCs are infected with *T. muris* and treated with a neutralizing monoclonal antibody to interferon- $\gamma$ , the production of T<sub>H</sub>2 cytokines is restored, which suggests that MHC class II-positive DCs can induce protective T<sub>H</sub>2 responses if the counter-regulatory T<sub>H</sub>1 response is blocked<sup>2</sup>. Thus, it seems that basophils are not strictly required for the initiation of T<sub>H</sub>2 responses. Instead, they promote T<sub>H</sub>2 differentiation by blocking DC-induced T<sub>H</sub>1 responses, at least during the development of *T. muris*-induced T<sub>H</sub>2 responses. Finally, basophil adoptive-transfer studies presented by both Sokol *et al.*<sup>1</sup> and Yoshimoto *et al.*<sup>3</sup> confirm that MHC class II-positive basophils are sufficient for the initiation of T<sub>H</sub>2 immunity *in vivo*.

Although the combined results from all three papers convincingly show that basophils can function as professional APCs and trigger T<sub>H</sub>2 differentiation both *in vitro* and *in vivo*, studies over the past 15-20 years suggest that a variety of mediators, cell types and mechanisms are involved in the development of polarized CD4<sup>+</sup> T<sub>H</sub>2 cell responses. Consequently, it will be necessary to determine whether all antigen-specific T<sub>H</sub>2 responses are initiated by this basophil-dependent mechanism or whether specific DC subsets or other APC populations trump basophils in some circumstances. In the studies presented here, basophils trump DCs because in addition to functioning as professional APCs, they also produce the key T<sub>H</sub>2-differentiating cytokine IL-4. Future studies will need to determine whether IL-4-producing, MHC class II-positive basophils are required simply for the initiation of T<sub>H</sub>2 responses or whether they are also critical in the maintenance of chronic T<sub>H</sub>2 responses. This information will be particularly useful because it might indicate whether targeting basophils would be beneficial in the treatment of persistent T<sub>H</sub>2-mediated diseases such as allergy and asthma. It would also be helpful to understand how basophils recognize specific allergens, proteases and parasite products and how these mediators trigger IL-4 production. Intravital imaging of basophil-T cell interactions in the lymph node in real time, as has been done with DCs, may also show how and when basophils are recruited to the draining lymph node during the initiation of an antigen-specific immune response. In conclusion, although the enigmatic basophil has been widely ignored by immunologists, the discovery that they can function as professional

APCs will probably create a flurry of interest and lead to new and exciting findings about their function in the regulation of disease.

## References

1. Sokol CL, et al. *Nat. Immunol* 2009;10:713–720. [PubMed: 19465907]
2. Perrigoue JG, et al. *Nat. Immunol* 2009;10:697–705. [PubMed: 19465906]
3. Yoshimoto T, et al. *Nat. Immunol* 2009;10:706–712. [PubMed: 19465908]
4. MacDonald AS, Straw AD, Bauman B, Pearce EJ. *J. Immunol* 2001;167:1982–1988. [PubMed: 11489979]
5. Seder RA, Paul WE, Davis MM, Fazekas de st Groth B. *J. Exp. Med* 1992;176:1091–1098. [PubMed: 1328464]
6. Moqbel R, et al. *J. Immunol* 1995;155:4939–4947. [PubMed: 7594499]
7. Ying S, et al. *J. Immunol* 1997;158:3539–3544. [PubMed: 9120316]
8. Brunner T, Heusser CH, Dahinden CA. *J. Exp. Med* 1993;177:605–611. [PubMed: 8436904]
9. Fort MM, et al. *Immunity* 2001;15:985–995. [PubMed: 11754819]
10. Pesce J, et al. *J. Clin. Invest* 2006;116:2044–2055. [PubMed: 16778988]
11. Liu YJ, et al. *Annu. Rev. Immunol* 2007;25:193–219. [PubMed: 17129180]
12. Rank MA, et al. *J. Allergy Clin. Immunol* 2009;116:2044–2055.
13. Pulendran B, et al. *Proc. Natl. Acad. Sci. USA* 1999;96:1036–1041. [PubMed: 9927689]
14. Maldonado-Lopez R, et al. *J. Exp. Med* 1999;189:587–592. [PubMed: 9927520]
15. Sokol CL, Barton GM, Farr AG, Medzhitov R. *Nat. Immunol* 2008;9:310–318. [PubMed: 18300366]
16. Jung S, et al. *Immunity* 2002;17:211–220. [PubMed: 12196292]
17. Lemos MP, Fan L, Lo D, Laufer TM. *J. Immunol* 2003;171:5077–5084. [PubMed: 14607905]



**Figure 1.**

A new paradigm for the initiation of type 2 immunity. In the present model (bottom), DCs serve as the main professional APCs for the development of antigen-specific CD4<sup>+</sup> T cell responses. During the development of TH1 and TH-17 responses, DCs are activated in the periphery by various pattern-recognition receptors (PRR) and migrate to the draining lymph nodes, where they present antigen to naive T cells in the context of MHC class II (MHC II). This 'DC<sub>1</sub>' population secretes specific cytokines, such as IL-12, that 'instruct' CD4<sup>+</sup> TH1 responses or cytokines such as IL-1, IL-6 and IL-23, which participate in the differentiation of TH-17 cells. In contrast to TH1- and TH-17-promoting antigens, TH2-inducing allergens, antigen-IgE immune complexes and helminth-derived secreted proteins activate an alternative APC-designated 'DC<sub>2</sub>' that requires an exogenous source of IL-4 to direct TH2 development. In the revised model (top), DCs are not required for the development of antigen-specific TH2 cell responses, because basophils can function as professional APCs. In contrast to the 'DC<sub>2</sub>' population, basophils also produce IL-4 when stimulated by TH2-inducing antigens in the draining lymph nodes. Consequently, accessory cells are no longer required for the initiation of TH2 responses in this model. TGF, transforming growth factor.