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Basophil-Macrophage Dialog in Allergic Inflammation

Yu Hui Kang¹ and Subhra K. Biswas^{1,*}

¹Singapore Immunology Network (SiGN), Agency for Science, Technology & Research (A*STAR), #04-01 Immunos, 8A Biomedical Grove, Singapore 138648, Singapore

*Correspondence: subhra_biswas@immunol.a-star.edu.sg
<http://dx.doi.org/10.1016/j.immuni.2013.02.015>

Monocyte-macrophage differentiation under pathological conditions is poorly understood. In the present issue of *Immunity*, Egawa et al. (2013) report how basophils drive the differentiation of inflammatory monocytes into M2 macrophages, thereby regulating allergic skin inflammation.

Monocytes and macrophages can perform a diverse range of functions under homeostatic and pathogenic conditions. This is facilitated by the existence of distinct subsets with specific functions and the ability to switch between different functional phenotypes in response to microenvironmental cues. Two main monocyte subsets have been recognized, namely the Ly6C^{hi}CCR2⁺ and Ly6C^{lo} CX3CR1⁺ monocytes in mice and the corresponding CD14⁺CD16[−] and CD16⁺CD14^{dim} monocytes in humans (Geissmann et al., 2010). The Ly6C^{hi}CCR2⁺ “inflammatory” monocytes infiltrate inflamed tissue, produce inflammatory cytokines, and are involved in host response to infection. The Ly6C^{lo}CX3CR1⁺ monocytes “patrol” blood vessels under steady state, extravasate during tissue injury, and promote tissue repair and healing. Similarly in macrophages, at least two main types of functional activation states exist: the M1 (or classically activated) and the M2 (or alternatively activated) macrophages. Microbial stimuli and T helper 1 (Th1) cell-related cytokines (such as IFN- γ) trigger the M1 phenotype, which promotes inflammation, Th1 cell responses, and microbicidal-tumoricidal functions. Th2 cell-related cytokines (such as interleukin-4 [IL-4], IL-13, and

IL-10) trigger the M2 state, which dampens excessive inflammation and promotes Th2 cell responses, tissue remodeling, protumor functions, and clearance of parasites (Biswas and Mantovani, 2010). However, M1 and M2 represent two extremes of a spectrum of macrophage functional states that may exist in vivo.

A key question arising from the above context is the relationship between the monocyte subsets and the macrophage activation states. In addition, the in vivo microenvironmental cues and interacting cells that induce monocyte-macrophage differentiation to distinct functional phenotypes, especially under pathological settings, is poorly understood. Addressing these areas, Egawa et al. (2013) in the present issue of *Immunity* investigate monocyte-macrophage differentiation in immunoglobulin E (IgE)-mediated skin allergic inflammation (IgE-CAI), uncovering a crucial role for basophils therein (Figure 1).

In mice sensitized with allergen-specific IgE, IgE-CAI was characterized by late-phase ear swelling after intradermal allergen challenge and was dependent on basophils. The skin lesions showed increased infiltration of Ly6C⁺CCR2⁺ monocyte-macrophages

and CCR2⁺ basophils and expression of CCR2 ligands CCL8 and CCL12. Based on these observations, a key role for CCR2 in the recruitment of these cells and the development of IgE-CAI was suggested. Contrary to this expectation, *Ccr2*^{−/−} mice showed exacerbated IgE-CAI with abolished monocyte-macrophage infiltration in the skin lesion but enhanced infiltration of basophils (as well as neutrophils), suggesting CCR2 to be dispensable for basophil recruitment. Further investigation profiling monocyte-macrophages from IgE-CAI skin lesions of wild-type mice revealed that the majority of these cells expressed the inhibitory molecule and M2 marker, Programmed Death 1 ligand 2 (PD-L2), together with other M2 genes like *Arg1*, *Chi3l3*, and *Fizz1*, indicating an M2 phenotype (Figure 1). Experiments via adoptive transfer of CD115⁺ bone marrow monocytes or inflammatory monocytes confirmed that inflammatory monocytes infiltrate skin lesions and differentiate into M2 macrophages, which in turn dampened the allergic inflammation, as demonstrated by their ability to attenuate the exacerbated IgE-CAI in *Ccr2*^{−/−} animals.

Several scenarios have emerged on the relationship between monocyte subsets

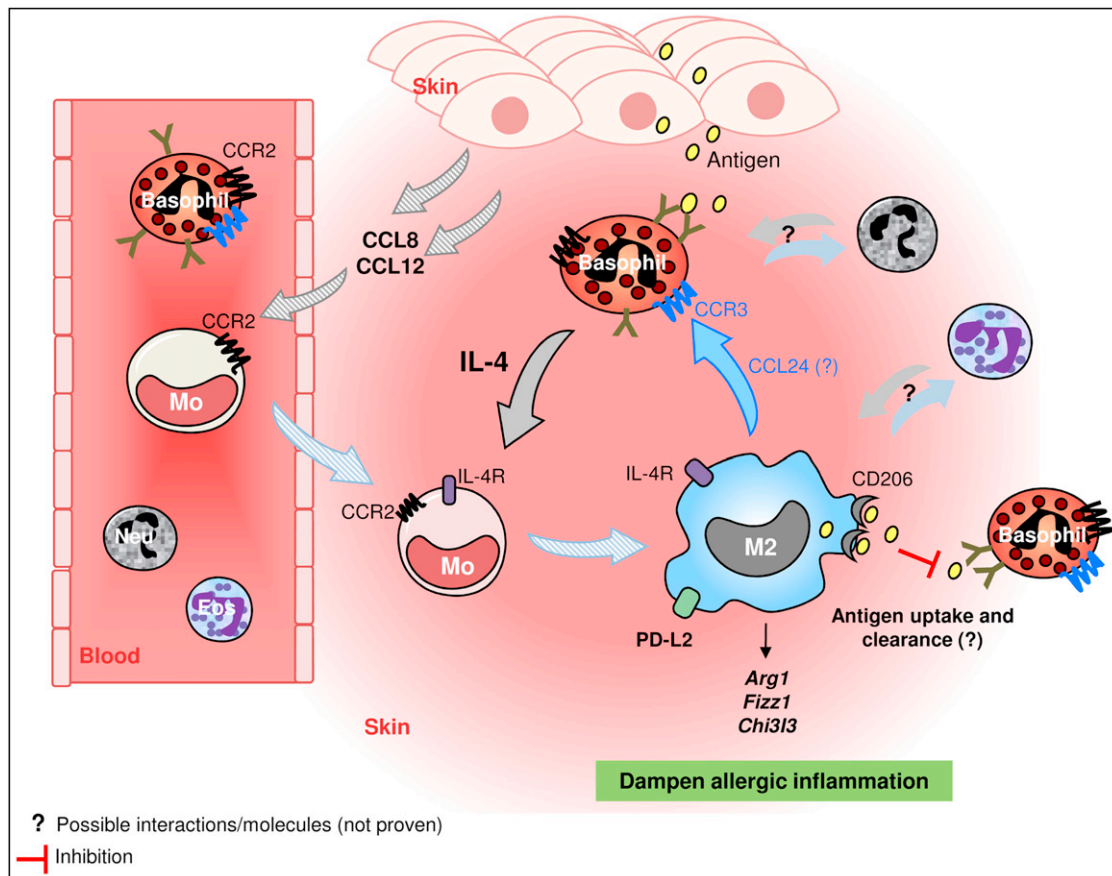


Figure 1. Monocyte Macrophage Differentiation and Interaction with Basophils in Allergic Skin Inflammation

During IgE CAI, circulating CCR2⁺Ly6C⁺ inflammatory monocytes (Mo) are recruited in response to upregulation of CCR2 ligands like CCL8 and CCL12 in the skin lesion. These Mo differentiate into M2 macrophage under the influence of IL-4 derived from activated basophils. These M2 macrophages, in turn, dampen allergic inflammation. M2 macrophages possibly mediate this via upregulation of mannose receptor, CD206, which facilitates efficient uptake and clearance of antigens, making it unavailable for basophil activation, thus attenuating allergic inflammation. Eosinophils (Eos) and neutrophils (Neu) are also recruited to IgE CAI skin lesions.

and their differentiation into polarized macrophages in pathological settings. During infection, inflammatory monocytes infiltrate the inflamed tissue and differentiate into M1 macrophages or inflammatory DCs (Geissmann et al., 2010). In contrast, patrolling monocytes, which infiltrate the inflamed tissue, ultimately differentiate into M2-like macrophages (Geissmann et al., 2010). In tumors, inflammatory monocytes differentiate into MHC-II^{hi} M1-like or MHC-II^{lo} M2-like macrophages depending on their location within the tumors and also migrate to metastatic sites giving rise to tumor-promoting, metastasis-associated macrophages (Biswas and Mantovani, 2010; Qian et al., 2011). In a helminth infection model, M2 macrophages were maintained via in situ proliferation, rather than by recruitment from blood mono-

cytes (Jenkins et al., 2011). A different situation is presented by the Egawa et al. (2013) study where inflammatory monocytes differentiate into M2 macrophages to suppress allergic inflammation. These contrasting examples indicate that the differentiation of monocyte subsets into polarized macrophages is influenced by several parameters such as the type and stage of disease, the subset(s) involved, the microenvironmental cues, and even the location within the tissue. In humans, the two main monocyte subsets were shown to differentiate in vitro into phenotypically distinct macrophages although their relationship to specific tissue macrophages remains unsettled (Frankenberger et al., 2012). The scenario is different in the steady state where major tissue-resident macrophages were reported to originate from yolk sac or fetal

liver progenitors and were maintained independently of monocyte input.

A key observation of the Egawa et al. (2013) study is the unexpected interaction between basophils and monocyte-macrophages (Figure 1). Basophils were the principal IL-4 producers in the allergic skin lesions. Results from in vitro experiments, in vivo genetic depletion of basophils, and adoptive transfer of CD115⁺ monocytes from *Il4ra*^{-/-} mice showed basophil-derived IL-4 to induce the M2 polarization of monocyte-macrophages (via IL-4 receptor) in the IgE-CAI skin lesions. IL-4 derived from Th2 cells and eosinophils induce M2 macrophages in helminth infection, lean adipose tissues, and tumors (Biswas and Mantovani, 2010; Murray and Wynn, 2011), but a basophil-macrophage crosstalk was not described. Concurring with the

present study, IL-4 derived from basophil progenitors in Lin[−]SHIP-deficient bone marrow cells were reported to mediate IL-3-induced M2 macrophage differentiation in mice (Kuroda et al., 2009). Basophils also produce thymic stromal lymphopoietin (TSLP), which amplifies M2 polarization in allergic airway inflammation (Han et al., 2013). Activated basophils release histamine, which may induce macrophages to express the M2-related chemokine CCL22. Moreover, M2 macrophages can release CCL24, which is a basophil chemoattractant, whereas activated basophils produce CCL3, CCL4, and CCL5, which can attract monocyte-macrophages, favoring their dialog (Figure 1; Biswas and Mantovani, 2010). Importantly, basophils are a main source for early IL-4 production in allergy and a possible initiator of Th2 cell-mediated responses (Sokol and Medzhitov, 2010). This raises the question as to whether basophils display distinct temporal roles in Th2 cell-mediated inflammation, i.e., initiating Th2 cell response in the early stage and attenuating it through M2 macrophages in the later stage. Further work will clarify this and whether the basophil-macrophage interactions described here is a general paradigm or specific to particular Th2 cell-related situations.

How M2 macrophages dampen IgE-CAI was not clear from the present

study. Although the contribution of arginase-1, PD-L2, or immunosuppressive cytokines was not favored, efficient antigen uptake and clearance by M2 macrophages making antigen unavailable for basophil activation was suggested. Another possibility arising from the observation that basophils and neutrophils show enhanced recruitment in *Ccr2*^{−/−} skin lesions is whether M2 macrophages themselves may block the recruitment of these cells, thereby attenuating Th2 cell-mediated inflammation. Increased expression of chemokines like CCL24 or CXCL8 by M2 macrophages (or basophils) may down-regulate and desensitize their cognate receptors on target cells, blocking their recruitment.

In summary, the present study provides an insight into monocyte-macrophage differentiation during allergic skin inflammation demonstrating inflammatory monocytes to differentiate into M2 macrophages, which then dampen IgE-CAI. Importantly, the M2 skewing of macrophages was mediated by basophils, emphasizing that basophil-macrophage crosstalk presents a unique paradigm in macrophage polarization, which merits further investigation. Finally, it is tempting to speculate whether modulating basophil activation may be a potential strategy to “reprogram” aberrant macrophages in disease settings,

including cancer, where polarized macrophages have an ambivalent role.

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HIV-1 Vaccines: Let's Get *Physical*

Nilu Goonetilleke¹ and Andrew J. McMichael^{1,*}

¹Weatherall Institute of Molecular Medicine, John Radcliffe Hospital, Oxford OX3 9DS, UK

*Correspondence: andrew.mcmichael@ndm.ox.ac.uk

<http://dx.doi.org/10.1016/j.immuni.2013.02.017>

Ferguson et al. (2013) use applied physics to quantitate the fitness of HIV-1 Gag based on sequence variability across the protein. This enables a new approach to vaccine design that focuses CD8⁺ T cell responses on fitness-constrained parts of Gag.

Considerable effort has been put into defining the immune responses induced by HIV-1 infection that are associated with greatest viral control, with the aim of reproducing the most effective

responses by prophylactic vaccination. Human leukocyte antigen (HLA) Class I proteins determine the specificity of CD8⁺ T cell responses and, given that certain HLA alleles are enriched in individ-

uals who exhibit marked HIV-1 control (elite controllers), the specificity of these T cell responses must be important for virus control. HIV-1 CD8⁺ T cell responses are first observed in acute