MHC-II: A Mutual Support System for ILCs and T Cells?

Rick M. Maizels[1,*] and David R. Withers[2]
[1]Institute of Immunology and Infection Research, and Centre for Immunity, Infection and Evolution, School of Biological Sciences, University of Edinburgh, Edinburgh EH9 3JT, UK
[2]MRC Centre for Immune Regulation, Institute for Biomedical Research, College of Medical and Dental Sciences, University of Birmingham, West Midlands B15 2TT, UK
*Correspondence: rick.maizels@ed.ac.uk
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Innate and adaptive immune cells form an ongoing partnership during an immune response. In this issue of *Immunity*, Oliphant et al. (2014) show that MHC class II-peptide presentation by group 2 innate lymphoid cells is needed for reciprocal regulation of both cell types, resulting in effective antihelminth immunity.

Many immunology students are taught that the innate immune system acts as our first rough-and-ready line of defense against infection, before the more finely honed adaptive B and T cell populations take over. Implicit in this model is that innate responses simply die out once adaptive immunity kicks in, but little consideration has been given to any mechanistic pathways capable of checking innate responsiveness.

Now, however, we are coming to appreciate that there is an ongoing innate-adaptive partnership in the immune response and that neither arm functions properly without the other. Thus, innate populations can fulfill critical roles, such as cytokine production, in the mature immune response alongside the adaptive cells. New data from Oliphant et al. (2014) reveal that for a subset of innate lymphoid cells (ILCs) and antigen-specific CD4^{+} T cells, this cooperation and reinforcement is mediated through major histocompatibility complex (MHC) class II interactions. This finding also illuminates a pathway through which continuing ILC activation is mediated, or indeed terminated, in the absence of appropriate peptide ligand or cognate T cells (Figure 1).

Although the initial studies characterizing cells that would become part of the ILC family noted their expression of MHC-II (Mebius et al., 1997, Neill et al., 2010), what functional role this served has been unclear. It is established that induction of T cell responsiveness is highly dependent on dendritic cells (Hammad et al., 2010, Phythian-Adams et al., 2010). Recently, it was demon-

strated that expression of MHC-II by the RAR-related orphan nuclear receptor γ (RORγ)-expressing group 3 ILC (ILC3) subset confers control of CD4^{+} T cell responses to commensal bacteria (Hepworth et al., 2013) and that MHC-II-expressing ILC2 could present peptide to T cells in vitro (Mirchandani et al., 2014). The latter authors also found that exogenous interleukin-2 (IL-2) stimulated cytokine production by ILC2s. Now, Oliphant et al. bring together these findings by showing that the stimuli ILC2s require from the T cells are only delivered upon recognition of cognate peptide presented by MHC-II. Such a mechanism allows for the decay of ILC2 activation once antigen from pathogens or other target sources has fallen below an effective threshold.

Understanding the roles of ILCs in vivo, within an intact immune system, has been hampered by a lack of robust mouse models. Oliphant et al. now report on two elegant strategies to selectively deplete ILCs in vivo. First, they target the inducible T cell costimulator (ICOS) locus to insert a diphtheria toxin receptor (DTR) subunit that would allow timed deletion of ICOS^{+} cells by administration of diphtheria toxin (DT) in vivo. Because ICOS expression is shared with many T cells, the DTR-encoding gene is flanked by Loxp sites that allow the insert to be excised in cells expressing *Cre*, in this case driven by the T cell-specific *Cd4* promoter. Hence, only ILCs will be liable to DT-mediated elimination. Second, the authors take advantage of the essential requirement for the RORα transcription factor in ILC development;

because this plays many other critical physiological roles, it is necessary to delete the gene only in lymphoid cells, which is accomplished by constructing a *Loxp3*-flanked RORα transgene and introducing *Cre* under the *Il7r* promoter (in this instance the *Rora*[fl] allele is paired with a functional knockout allele, *Staggerer*, to maximize the efficiency of deletion). Both systems showed a dramatic reduction in CD4^{+} T cell type 2 responses, measured by IL-5 and IL-13 production, and in expulsion of the intestinal helminth *Nippostrongylus brasiliensis*, indicating the need for innate ILC2s in amplifying the adaptive type 2 response.

In considering potential mechanistic interactions between ILC2s and T cells, Oliphant et al. assessed MHC-II expression by ILC2s, which was expressed in significantly lower and more heterogenous amounts than on B cells, and expression was lost following short-term culture; the unusual plasticity of MHC-II expression on ILCs does call into question whether they constitute a dedicated antigen-presenting cell. On the other hand, many ILC2s also expressed CD80 and CD86, suggesting that they are able, in the appropriate setting, to drive T cell activation. This is in contrast to the ILC3 subset, which lacks expression of these costimulatory molecules, consistent with a regulatory role in tolerizing T cell responses (Hepworth et al., 2013). The authors show that ILC2s, in the absence of dendritic cells (DCs), will drive proliferation of T cell receptor (TCR) transgenic T cells in vitro and that, furthermore ILC2s can take up, process, and present

antigen in a manner analogous to professional APCs. They also suggest that the low MHC-II expression on ILC2s might actually correspond to the selective differentiation of T helper 2 (Th2) cells, in that reduced ligand density and TCR engagement is thought to predispose in that direction (van Panhuys et al., 2014). However, it would seem to be early days in judging whether ILC2s play any critical role in antigen presentation or are simply endowed with the ability to top up the T cell response when the professionals are otherwise engaged.

If ILC2s use MHC-II to present antigen, what happens when MHC-II-deficient ILC2s are administered to mice in vivo? The authors tested this in a transfer system in which recipient mice are IL-13-deficient and thereby unable to expel *N. brasiliensis*. When wild-type (MHC-II+IL-13+) ILC2s are transferred, parasites are expelled; however, if the ILC2s lack MHC-II, the recipients fail to expel despite a vigorous Th2 response due to endogenous MHC-II+ APCs. Thus, the tables are turned and MHC interactions are required to activate (or to maintain the activation of) ILC2s for IL-13 production, clearly demonstrating for the first time that ILC2:CD4+ T cell crosstalk potentiates the innate type 2 response.

In vitro cocultures established that MHC-II antibody blockade affected not only T cell activation but also the stimulation of proliferation and cytokine production by ILC2s. This result provides confirmation that MHC-II acts as a display on ILCs that allows T cell scrutiny and regulation. But does this checkpoint operate by ILCs being dependent on a surface-mediated interaction with T cells, or on soluble cytokines? Part of the answer at least is through soluble mediators, with the key cytokine responsible shown to be IL-2, both in vitro (modulating ILC2 expression of IL-13
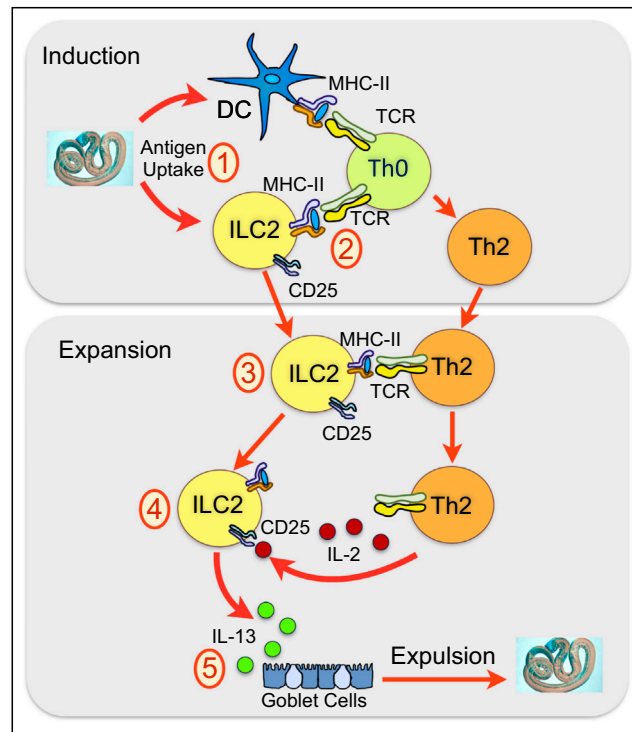

Figure 1. The Continuing Interplay between ILC2s and CD4+ T Cells
The expression of MHC-II by ILC2s and their ability to endocytose and process antigen (1) might allow them to present peptide to and activate naive Th0 cells (2), possibly favoring differentiation to Th2 by low ligand density. Once induced (by conventional DCs as well as ILC2s), Th2 cells subsequently interact with ILC2s, inspecting their MHC-II cargo, which, if bound by the TCR, gives a further activation signal to the ILC2s (3), including the release of IL-2 that ligates CD25, the high-affinity IL-2 receptor (4). These signals drive further IL-13 production by ILC2s, orchestrating a suite of innate effector cells such as mucus-producing goblet cells (5), as well as alternatively activated macrophages (not shown) that promote expulsion of intestinal helminths.

by adding or blocking IL-2) and in vivo (in recombinase-activating-gene-deficient mice in which limiting IL-2 is available due to the absence of T cells). In the latter model, the authors succeeded in inducing worm expulsion by adding IL-2 to the ILCs, which otherwise cannot achieve functional immunity.

However, this result implies that whereas ILC2s present peptide to T cells, which only produce IL-2 if there is a cognate interaction with their TCR, the IL-2 produced can activate all ILC2s irrespective of their peptide loading. Arithmetically, this might be an efficient mechanism at a time point at which the antigen-specific Th2 cells have greatly expanded (so that there are plenty of cognate T cells whose job it is to produce IL-2) and a relatively small pool of ILC2s are recruited to the maximum possible extent, regardless of whether they have

taken up a particular antigen. It will be interesting to observe whether further mechanisms of crosstalk between ILC2s and T cells are identified, perhaps through cell-surface receptor-ligand interactions. For the MHC-II+ ILC3s at least, the expression of a distinct set of costimulatory molecules suggests that additional ILC:T cell interactions influence the response (Kim et al., 2003).

What are the broader implications of these findings? Oliphant et al. have developed refined mouse models that will enable the dissection of ILC2 function in vivo, particularly the contribution of these cells to adaptive immune responses. There is now a stronger emphasis placed on ILC2s as the source of functional IL-13 in the longer term during an immune response, but the ongoing production of this cytokine is dependent on approval by CD4+ T cells, as if the adaptive immune system is simply outsourcing the heavy lifting to a less sophisticated population. The studies have tested functionality in the helminth system of *N. brasiliensis*, which is a relatively soft target in being readily expelled by immunosufficient mice: it might be interesting to test the ability of IL-2-driven ILC2s to act autonomously in a more challenging infection such as *Schistosoma mansoni* or *Heligmosomoides polygyrus* and even more interesting to evaluate their contribution in allergic models such as asthma-like inflammation of the airways. Notably, ILC2s in secondary lymphoid tissue express higher amounts of MHCII than in tissues such as the lung and gut (Hepworth et al., 2013), perhaps indicating activation states for ILC2s. Identification of how MHC-II is regulated on ILCs appears to be a key question for the future. If ILC2s, spurred on by continual dialog with T cells, are responsible for the longer-term exacerbation and disease in the asthma setting, they will present a vital target for novel therapies yet to be developed.

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"Nuts and Bolts" of Disease Tolerance

Miguel P. Soares[1],*
[1]Instituto Gulbenkian de Ciência, 2780-156 Oeiras, Portugal
*Correspondence: mpsoares@igc.gulbenkian.pt
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Disease tolerance describes the ability of an infected host to limit disease severity without negatively impacting the causative pathogen. Bessede et al. (2014) show that the aryl hydrocarbon receptor is an essential component of disease tolerance during bacterial infection in mice.

The pathologic outcome of infection is revealed by the appearance of clinical symptoms, reflecting a more or less pronounced dysfunction of homeostasis in the infected host. Depending on the severity of disease, host reproductive capacity and survival—fitness—might eventually be compromised as well. It follows that host defense strategies against infection should share as a common endeavor the preservation of homeostasis and fitness. The prevailing strategy to achieve this goal is to eliminate the causative agent of disease, i.e., the pathogen, via immune-driven resistance mechanisms (Figure 1).

Host resistance mechanisms rely on the recognition of pathogens by germline-encoded pattern recognition receptors (PRR), activating the host innate immune system, which targets pathogens for destruction and/or expulsion (Figure 1). Activation of adaptive immunity provides a more specific, robust, and long-lasting protection mechanism against infection. Enhancing immune-driven resistance mechanisms, e.g., through vaccination, has proven to be an extremely efficient therapeutic strategy against infectious diseases, relieving

mankind from the evolutionary constraints imposed by many pathogens. Presumably for this reason, we came to consider immune-driven resistance mechanisms as the only defense strategy that really matters when taking into consideration protection against infectious diseases. Reality, however, is probably more complex.

The study by Bessede et al. (2014) highlights the "relative cost" associated with immune-driven resistance mechanisms, as these become pathologic and contribute to disease severity, i.e., immunopathology (Figure 1). Bessede et al. (2014) show that this evolutionary trade-off is reduced via an immunoregulatory mechanism involving a stress-response pathway controlled by the aryl hydrocarbon receptor (AhR) and conferring disease tolerance to infection (Figure 1).

Disease tolerance is a concept that stems from observations made originally in the context of infection in plants and revealing that these can "tolerate" pathogens via a defense strategy that does not appear to reduce their pathogen load but instead limits the extent of tissue damage associated with infection (Schaefer,

1971). This defense strategy, coined as tolerance, remained in the literature for more than a century, as a specificity of host-pathogen interactions in plants (Schaefer, 1971). As it turns out, however, tolerance is an evolutionary conserved host defense strategy against infection that is shared by plants and animals, including insects, worms, mice, and most likely humans as well (Medzhitov et al., 2012). Disease tolerance is the term used to describe the same concept defined originally in the plant literature and referring to preservation of host fitness during infection, without concomitant reduction of pathogen load (Medzhitov et al., 2012). The mechanisms underlying disease tolerance in mammals remain poorly understood, being linked so far to stress-responsive pathways that limit the extent of tissue damage caused directly by pathogens or indirectly by host immune-mediated resistance mechanisms (Figure 1; Figueiredo et al., 2013; Jamieson et al., 2013; Larsen et al., 2010). Bessede et al. (2014) propose that the stress-response pathway regulated by AhR is critically involved in promoting disease tolerance to bacterial infections in mice.