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Programming dendritic cells to induce T_H2 and tolerogenic responses

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A fundamental puzzle in immunology is how the immune system decides what types of immune responses to launch against different stimuli. Although much is known about control of T helper type 1 (T_H1) and T_H17 responses, the mechanisms that initiate T_H2 and T regulatory (T_{reg}) responses remain obscure. Emerging studies suggest a fundamental role for the innate immune system, particularly dendritic cells (DCs), in this process. We review these studies, and suggest that the innate control of T_H2 and T_{reg} responses can be viewed as different hierarchies of organization, in which DCs, their innate receptors and signaling networks, and their interactions with other cells and local microenvironments represent different levels of the hierarchy.

Encounter with a microbe poses several decision-making challenges to the immune system. To respond, or not to respond? If making a response, then what type should it be? Indeed, a hallmark of the immune system is its ability to induce distinct types of responses against different classes of pathogens. Twenty-five years ago, Coffman and Mosmann made the seminal observation that CD4^{+} T helper (T_H) cell clones can be divided into two classes, T_H1 and T_H2, on the basis of their cytokine profiles[1]. Interferon-γ (IFN-γ)-secreting T_H1 cells are effective at combating intracellular bacteria and viruses, by means of the activation of macrophages and NK cells and expansion of cytotoxic T cells (CTLs). In contrast, T_H2 cells, whose cytokines (interleukin (IL)- 4, IL-5 and IL-13) direct immunoglobulin E (IgE)- and eosinophil-mediated destruction of pathogens, are effective at controlling helminths[2]. Although helminths are considered the prototypic T_H2-inducing stimuli, bacteria, viruses and allergens can also induce T_H2 responses. More recently a third subset of T_H cells, the T_H17 subset, has been discovered. T_H17 cells produce IL-17A, IL-17F and IL-22 and are thought to be key in immunity against extracellular bacteria and fungi[3]. At around the same time, it was demonstrated that naive CD4^{+} T cells could be induced to differentiate into Foxp3^{+} T regulatory (T_{reg}) cells by T cell antigen receptor (TCR) stimulation in the presence of transforming growth factor (TGF)-β and IL-2 (ref. 4). T_{reg} cells regulate T_H1, T_H17 and T_H2 responses, which if unchecked can lead to inflammatory disorders such as autoimmunity (T_H1, T_H17 responses) and allergy (T_H2 responses). T_{reg} cells are also important in maintaining immune tolerance against self antigens[2,5], and pathogens have evolved strategies that induce T_{reg} cells, thereby evading the host immune response while preventing immunopathology, thus creating a state of détente[5]. The differentiation of these T_H cells is regulated by distinct transcription factors (T-bet and signal transducer and activator of transcription (STAT)-4 for T_H1 cells, GATA3 and STAT5 for T_H2 cells, RORγt and STAT3 for T_H17 cells, and Foxp3 and STAT5

for T_{reg} cells)[2]. In addition to these subsets, other subsets have been identified, including IL-10-producing 'Tr1 cells'; TGF-β-producing 'T_H3 cells', which also suppress T_H1, T_H17 and T_H2 responses and induce immunological tolerance; IL-9-producing 'T_H9 cells'; and T follicular cells ('T_{FH} cells'), located within the B cell–rich follicles of lymphoid organs[2]. The lineage relationship between these subsets and T_H1, T_H2, T_H17 and T_{reg} cells are still being defined.

The capacity of distinct T_H responses to protect against different pathogens and the immunopathology that can develop from their unbridled activation place a great premium on understanding the cellular and molecular mechanisms that control such responses, with a view to the rational design of vaccines and therapeutics. Advances in immunology over the past decade have revealed a fundamental role for the innate immune system in sensing pathogens and tuning the quality of T_H responses. Although there has been much progress in understanding the role of innate immunity in inducing T_H1 and T_H17 responses[2], we understand very little about its role in initiating T_H2 and 'tolerogenic' (T_{reg}, Tr1 and T_H3 cell) responses. However, there are emerging insights into the roles of DC subsets, pathogen recognition receptors (PRRs), signaling pathways and accessory cell types that orchestrate T_H2 and tolerogenic responses. In the present Review, we will summarize these advances, highlight unanswered questions and offer a conceptual framework for understanding the innate control of T_H2 and tolerogenic responses.

Hierarchies of organization in the innate immune system
According to the classical model (**Fig. 1a**), DCs are activated by microbial stimuli signaling through PRRs, which then program them to induce distinct innate responses that shape the type of T_H response[6]. However, entry of a microbe into the body can also activate a range of other cell types, such as NK cells, NK T cells, basophils, mast cells, myeloid suppressor cells, T_{reg} cells, tissue epithelial cells and stromal cells, all of which influence DC function (**Fig. 1b**). Therefore, in addition to the direct DC activation by microbes, DCs orchestrate the concerted action of a network of cell types. Thus, a unified model of the cellular and molecular mechanisms that initiate and control T_H2
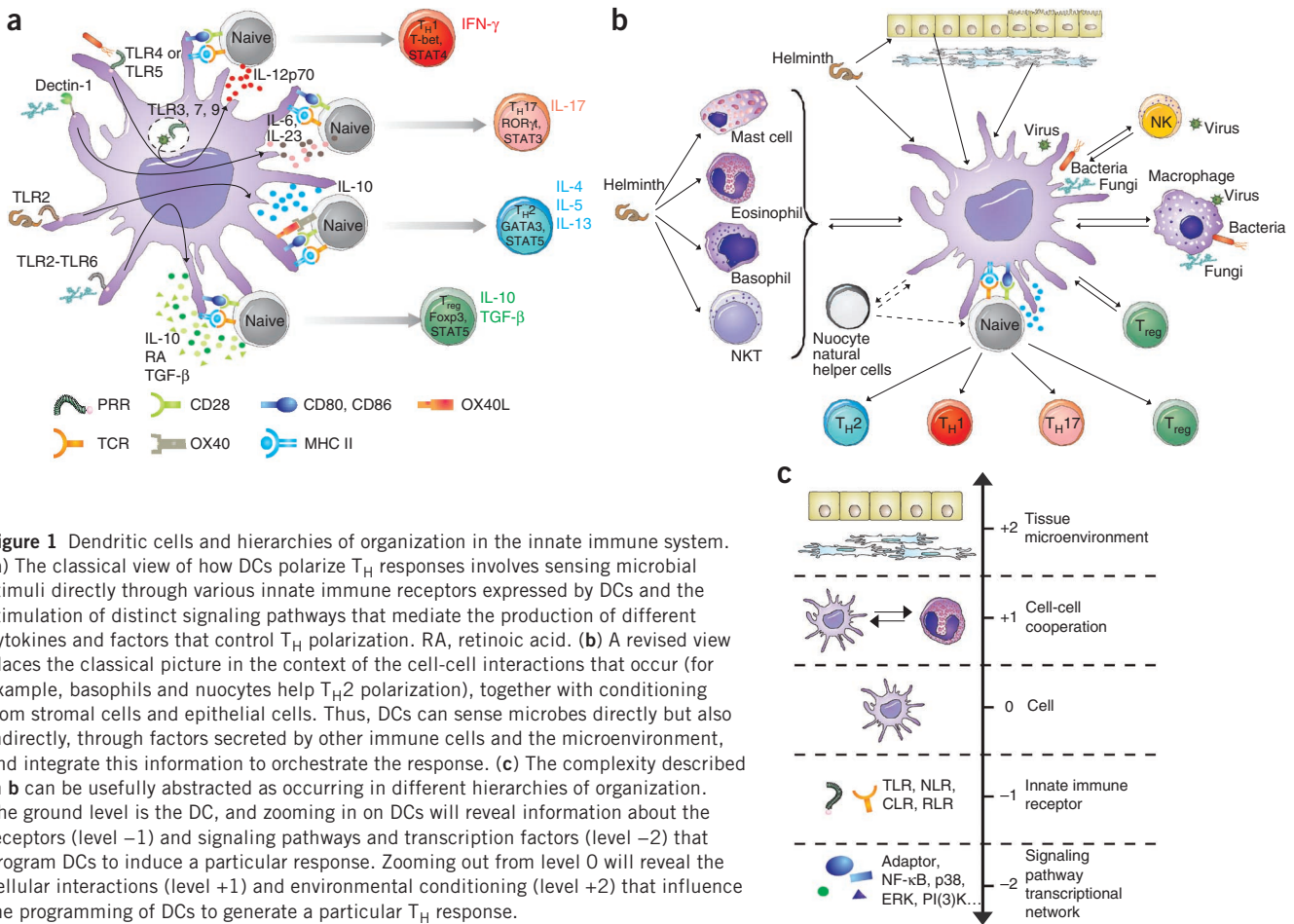
Figure 1 Dendritic cells and hierarchies of organization in the innate immune system. (**a**) The classical view of how DCs polarize T$_H$ responses involves sensing microbial stimuli directly through various innate immune receptors expressed by DCs and the stimulation of distinct signaling pathways that mediate the production of different cytokines and factors that control T$_H$ polarization. RA, retinoic acid. (**b**) A revised view places the classical picture in the context of the cell-cell interactions that occur (for example, basophils and nuocytes help T$_H$2 polarization), together with conditioning from stromal cells and epithelial cells. Thus, DCs can sense microbes directly but also indirectly, through factors secreted by other immune cells and the microenvironment, and integrate this information to orchestrate the response. (**c**) The complexity described in **b** can be usefully abstracted as occurring in different hierarchies of organization. The ground level is the DC, and zooming in on DCs will reveal information about the receptors (level −1) and signaling pathways and transcription factors (level −2) that program DCs to induce a particular response. Zooming out from level 0 will reveal the cellular interactions (level +1) and environmental conditioning (level +2) that influence the programming of DCs to generate a particular T$_H$ response.

and tolerogenic responses is likely to result from studying different hierarchies of organization in the innate immune system (**Fig. 1c**). The cell can be considered the 'ground level' of this hierarchy (level 0), and zooming in on the cell to examine innate receptors (hierarchy level −1) and signaling networks (hierarchy level −2) offers more detailed levels of abstraction. In contrast, zooming out from the cell allows more global views of multicellular cooperation (for example, between DCs and basophils or DCs and stromal cells—hierarchy level +1) and the influence of tissue microenvironments (for example, intestine versus lung—hierarchy level +2).

Hierarchy level 0: cells

DCs can prime T$_H$2 responses, and distinct DC subsets induce different T$_H$ responses (**Table 1**). For example, in mouse spleens there are two main phenotypically and functionally distinct DC subsets. CD11c$^+$CD8α$^+$DEC205$^+$ DCs found in the T cell–rich areas can be induced to produce copious IL-12p70. In contrast, CD11c$^+$CD8α$^-$DEC205$^-$ DCs are located in the marginal zones, and generally do not produce much IL-12p70, but can be induced to produce IL-10 (refs. 7,8). Consistent with this, adoptive transfer of antigen-pulsed CD8α$^+$ versus CD8α$^-$ mouse splenic DCs into mice differentially induces T$_H$2 versus T$_H$1 responses *in vivo*[7,8]. Furthermore, targeting of antigen to these subsets using specific antibody-antigen constructs results in induction of T$_H$1 versus T$_H$2 responses *in vivo*[9]. Resting respiratory tract DCs preferentially stimulate T$_H$2 cells[10], and myeloid DCs induce T$_H$2 responses to inhaled antigen, leading to eosinophilic airway inflammation[11]. In humans,

plasmacytoid DCs in the blood[12] and Langerhans cells in the skin[13] can preferentially induce T$_H$2 responses, and in some cases also induce T$_{reg}$ cells.

In addition to their functional specializations, DCs also show a great deal of functional plasticity. Thus, various microbial stimuli, including *Porphyromonas gingivalis* lipopolysaccharide (LPS)[14], fungal products[15,16], *Schistosoma mansoni* egg antigen (SEA[17]), Omega-1 (a T2 RNase glycoprotein derived from SEA)[18,19], helminths[20],

Table 1 Evidence that DCs induce T$_H$2 responses

Observation	References
Adoptive transfer of specific subsets of DCs (for example, mouse splenic CD8α$^-$ DCs) preferentially induces T$_H$2 responses *in vivo*. In humans, specific DC subsets induce T$_H$2 responses	7,8,10–13,84
Targeting antigen to specific DC subsets preferentially induces T$_H$2 responses	9
Specific microbial stimuli (for example, *P. gingivalis* LPS, fungal proteases, cholera toxin, SEA) program DCs to induce T$_H$2 responses	14–23,26
TSLP conditions DCs to induce T$_H$2 responses	28–30
Allergens and mediators of allergy program DCs to induce T$_H$2 responses	23–26,31,84
In vivo depletion of lung CD11c$^+$ DCs during allergen challenge abrogates asthma	32
Immune responses induced by the T$_H$2-inducing adjuvant alum are dependent on DCs	33

Table 2 Evidence for involvement of DCs in inducing tolerogenic responses

Observation	References
Immature DCs induce anergy of Tr1 cells or Foxp3+ T_{reg} cells Disruption of E-cadherin-mediated DC-DC interactions triggers an alternative pathway of maturation mediated by β-catenin and programs the DCs to a tolerogenic state	Reviewed in 6,48 49
Exposure of DCs to anti-inflammatory and immunosuppressive agents programs them to a tolerogenic state	Reviewed in 50,51
Capture of apoptotic cells or self antigens by DCs in the steady state programs them to a tolerogenic state	48,52,129
Specific microbial stimuli (for example, zymosan, *Y. pestis* virulence factor LcrV, phosphatidylserine from *S. mansoni*) program DCs to induce T_{reg} cells	56–58,75,76
Specific DC subsets in mucosal sites (for example, CD103+ DCs in intestinal lamina propria and mesenteric lymph nodes) are programmed to induce T_{reg} cells	60–62

cholera toxin[21], allergens[22–26] or prostaglandin E[27] can all program DCs to induce T_H2 responses. Importantly, the allergic mediators thymic stromal lymphopoietin (TSLP), which is produced by stromal cells[28–30], and histamine[31] can also condition DCs to induce T_H2 responses. Thus DCs can prime T_H2 responses; but evidence that they actually do comes from studies in which the conditional ablation of DCs *in vivo* using CD11c-diphtera toxin (DTR) mice abrogates allergen-induced asthma[32] and alum induced T_H2 responses[33]. In addition, T_H2 response to the cysteine protease papain is dependent on DCs and is mediated by dermal DCs[34].

A key issue is the nature of the molecules that induce T_H2 responses. *In vivo*, IL-4 is not always required for T_H2 polarization. The nematode *Nippostrongylus brasiliensis* induces T_H2 responses that are diminished, but not absent, in mice that are deficient in IL-4Rα or STAT6; but GATA3 remains essential. In contrast, the generation of T_H2 responses to the nematode *Trichuris muris* requires IL-4 (ref. 2). If IL-4 is required for T_H2 differentiation, what is the cellular source of the initial IL-4 production? As naive T cells do not produce IL-4, this raises the chicken-and-egg question of what induces T_H2 cells to make IL-4 in the first place. Basophils and mast cells promote T_H2 responses by rapidly producing IL-4 upon cross-linking of their FcεRI receptors, through preexisting antigen-IgE complexes[35–39]. This might seem paradoxical, because the production of such antibodies would have required the existence of primed CD4+ T_H2 cells specific to that antigen in the first place. However, microbial stimuli and allergens can directly stimulate basophils and mast cells to produce IL-4. Basophils are critical in priming T_H2 responses against helminths and protein allergens, and this process will be discussed in detail below[40–43]. CD4+NK1.1+ ('natural killer T', or NKT) cells also produce large amounts of IL-4 rapidly after TCR triggering[44]. However, mice lacking NKT cells, such as CD1d- or β2-microglobulin knockout mice, launch normal T_H2 responses, so these cells might not be essential contributors of IL-4 (ref. 45).

Apart from IL-4, are there other signals that induce T_H2 responses? One model posits that absence of IL-12p70 production by DCs results in a 'default' T_H2 response. Thus, *P. gingivalis* LPS, cholera toxin, SEA, TSLP and other stimuli condition DCs to induce T_H2 responses, in part by suppression of IL-12 in DCs[14–31]. An alternative model is that other molecules on DCs, such as OX40-l (ref. 29) and the Notch ligands Jagged-1 and Jagged-2 can induce T_H2 responses[46]. In addition, production of stromal or epithelial cell–derived mediators such as TSLP, IL-33 or IL-25 can exert potent influences on DCs and program them to induce T_H2 responses.

What are the roles of DCs in tolerogenic responses? Their importance in negative selection in the thymus and central tolerance was established by targeted expression of MHC class II molecules on DCs[47]. In the periphery, recent work has highlighted their role in suppressing immune responses and inducing immune tolerance (**Table 2**). The tolerogenic functions of DCs can depend on their maturation stage, anti-inflammatory and immunosuppressive agents, the nature of the microbial stimuli, and the tissue microenvironment[48]. Immature DCs that express low surface amounts of MHC and costimulatory molecules induce suboptimal T cell priming, often leading to T cell anergy or tolerance[6,48]. Interestingly, an alternative form of DC maturation, which is triggered by alterations in E-cadherin-mediated DC-DC adhesion, can occur under steady-state conditions[49]. Selective disruption of these interactions induces the typical features of DC maturation, including upregulation of co-stimulatory molecules, MHC class II and chemokine receptors. These events are triggered at least in part by activation of the β-catenin pathway. However, unlike DCs induced to mature by microbial products, E-cadherin-stimulated DCs do not release immuno-stimulatory cytokines, and they induce tolerogenic T cells[49].

In addition to their dependency on the maturation stage, tolerogenic DCs can also be induced by exposure to various anti-inflammatory and immunosuppressive agents, such as IL-10 and TGF-β1, inducers of cyclic AMP such as prostaglandin E2, the vitamin D3 metabolite 1α,25-dihydroxyvitamin D3 and its analogs[50]. Another mechanism by which DCs can promote tolerogenic responses involves tryptophan catabolism by indoleamine 2,3-dioxygenase (IDO)[51], which can be induced by certain Toll-like receptor (TLR) ligands or CTLA-4. Furthermore, DCs can promote tolerance upon capture of antigens from dying cells or of harmless self and environmental antigens[48,50,52,53]. A key role for DCs in inducing tolerance *in vivo* was initially noted in experiments involving targeting antigen into DCs through the endocytic receptor DEC-205 (refs. 48,54), which is expressed on CD8α+ DCs.

Microbes have evolved several strategies for programming DCs to induce T_{reg} cells. For instance, persistent interactions between microbes and hosts (as would occur during chronic infections, or in the commensal-rich intestine) could result in excessive inflammation and immunopathology, which is evolutionarily undesirable for the host or microbe. Therefore, microbes have evolved strategies to maintain immune homeostasis by inducing T_{reg} cells that control unbridled host immunity. For example, different fungal morphotypes (that is, conidia or hyphae) of *Candida albicans* induce different intracellular signaling which program DCs to stimulate distinct T_H responses[55]. Thus, conidia stimulate inflammatory DCs that initiate T_H17 or T_H2 responses, whereas hyphae programs DCs to activate T_H1 or T_{reg} cells[55]. The capacity to modulate the T_H17–T_{reg} cell balance might be exploited by the fungus to achieve commensalism or pathogenicity. Furthermore, phosphatidylserine and lysophosphatidylserines from *S. mansoni* condition DCs through TLR2 signaling to induce T_{reg} cells[56]. Filamentous hemagglutinin (FHA) from the bacteria *Bordetella pertussis* induces DCs to produce IL-10 and prime Tr1 cells[57]. *Yersinia pestis* is known to activate DCs by means of the dimer of TLR2 and TLR6 to induce T_{reg} cells[58].

Recent studies have highlighted the existence of tolerogenic antigen-presenting cells in mucosal environments. Given the enormous burden of microbial stimuli (>10^{14} commensal microorganisms in the intestine[59]), intestinal DCs and macrophages have regulatory mechanisms to prevent excessive inflammation. For instance, there is an active suppression of proinflammatory cytokine production by immunoregulatory cytokines, such as IL-10 (ref. 5). Intestinal DCs

Table 3 Innate receptors that control T$_H$2 or tolerogenic responses

Receptor	Comments	References
TLRs		
TLR2	Several ligands of TLR2 induce T$_H$2 or T$_{reg}$ responses	14,58,68–81
TLR4	Low doses of inhaled LPS signal through TLR4 to induce T$_H$2 responses to inhaled antigens; Der p2 functions as allergen because of its ability to bind LPS and mimic the function of MD2, a component of TLR4 complex; house dust mite allergen induces asthma through TLR4	83,84
NLRs		
NOD1 and NOD2	Innate sensing of peptidoglycan by Nod1 and Nod2 induces T$_H$2 responses	87,88
NALP3	Alum, a T$_H$2-inducing adjuvant, depends partly on the NALP3 inflammasome for its immunogenicity; however, the mechanism by which it induces T$_H$2 responses is unclear	89–91
CLRs		
DC-SIGN	Mycobacteria target DC-SIGN to suppress DC function; *H. pylori* Lewis antigen induces T$_H$2 responses through DC-SIGN; the allergen derived from *A. hypogaea* induces T$_H$2 responses through DC-SIGN	95,130
Fcγ receptors	Ligation of FcγRI and FcγRIII on DCs and macrophages inhibits TLR-mediated IL-12 production and programs the DCs to induce T$_H$2 responses	98,99
Complement receptors		
C5a, CR3	C5a and CR3 triggering negatively regulates TLR-mediated IL-12 production and biases toward T$_H$2 responses	101,102
Protease-activated receptors		
PAR-2	PAR-2 mediates induction of the T$_H$2-inducing cytokine TSLP in response to the fungus *A. alternata*; protease kallikrein-5 induces atopic dermatitis–like lesions, through PAR-2-mediated induction of TSLP	104,105

expressing CD103 can specifically induce Foxp3$^+$ T$_{reg}$ cell from naive CD4$^+$ T cells *in vitro* in a retinoic acid–dependent fashion[60,61]. In addition, lamina propria macrophages are hyporesponsive to various inflammatory stimuli[62], spontaneously secrete IL-10 and can efficiently promote Foxp3$^+$ T$_{reg}$ cell conversion *in vitro*[62]. IL-10 production by these lamina propria macrophages is responsible for maintaining Foxp3 expression in T$_{reg}$ cells during intestinal inflammation[63].

Like the intestine, the lung is also constantly exposed to various microbes and allergens. Emerging studies show that, like intestinal DCs, lung DCs also play important regulatory roles in response to inhaled inert antigens. As in the intestine, lung CD103$^+$ DCs promote the induction of T$_{reg}$ cells[64], and lung CD103$^-$ DCs represent the main producers of proinflammatory cytokines in response to airborne allergens or TLR ligands. In addition to conventional DCs, *in vitro* and *in vivo* studies show that lung plasmacytoid DCs also promote T$_{reg}$ cell induction[65]. In the skin, in contrast to the intestine and lung, CD103$^-$CD11b$^+$ migratory dermal DCs are much more potent in inducing Foxp3$^+$ T$_{reg}$ cells than are CD103$^+$CD11b$^+$ DCs[64].

Hierarchy level –1: innate receptors

DCs can sense pathogens and allergens directly by means of receptors of the innate immune system, such as TLRs, C-type lectin–like receptors (CLRs), RIG-I–like receptors (RLRs) and Nod-like receptors (NLRs)[66]. Whereas there is a considerable understanding of how microbial stimuli signal through such receptors to induce T$_H$1 responses, our understanding of the receptors that induce T$_H$2 or tolerogenic responses is still fragmentary. Helminths, bacteria, viruses and allergens are all capable of inducing T$_H$2 responses[67]. As might be expected, the immune system seems to have evolved a broad range of receptors to sense these diverse stimuli (**Table 3**).

TLRs are expressed on surface membrane or in endosomal compartments[66]. TLRs on the surface membrane recognize microbial stimuli such as LPS (TLR4), lipoteichoic acids of Gram-positive bacteria and bacterial lipoproteins (TLR1-TLR2 and TLR2-TLR6 dimers), and flagellin (TLR5), whereas endosomal TLRs mainly detect microbial nucleic acids, such as double-stranded RNA (TLR3), single-stranded RNA (TLR7) and CpG DNA (TLR9). Signaling through most TLRs induces T$_H$1 responses. For example, LPS, CpG DNA, poly(I:C) and TLR7 ligands induce IL-12p70 and IFN-α production in DCs and stimulate T$_H$1 responses[66]. Many TLR2 ligands also induce weak IL-12p70 production, and *Myd88$^{-/-}$* mice seem to have a selective

defect in T$_H$1 responses[66], suggesting that MyD88-dependent TLRs preferentially mediate T$_H$1 responses and not T$_H$2 responses. However certain TLR2 ligands can also induce T$_H$2 responses[14,56,68–74]. Signaling through TLR2 can also result in tolerogenic responses[75–81]. Stimulation of splenic DCs with specific TLR2-TLR6 ligands such as the yeast zymosan[75–77] or *Y. pestis* virulence factor LcrV induces T$_{reg}$ cells[58]. Consistent with this, TLR2 ligands on staphylococcal cell walls downregulate superantigen-induced T cell activation and prevent toxic shock syndrome, through the induction of IL-10 (ref. 82).

In addition to the anti-inflammatory effects of TLR2, low doses of LPS have also been to shown to induce T$_H$2 cells in response to intranasal immunization with protein antigens, by means of a TLR4- and MyD88-dependent mechanism[83]. Furthermore, extracts of house dust mite allergens (HDM extracts, which are known to contain LPS) signal through TLR4 on airway structural cells to produce cytokines such as TSLP, granulocyte monocyte colony stimulating factor (GM-CSF), IL-25 and IL-33, which condition DCs to promote T$_H$2 response and airway inflammation[84]. Consistent with this, the main HDM allergen Der p 2 is structurally and functionally homologous to MD-2 (the LPS-binding component of the TLR4 complex) and directly interacts with TLR4 to facilitate TLR4 signaling T$_H$2-mediated allergic inflammation[85]. Interestingly, the cysteine protease papain induces T$_H$2 responses *in vivo*, through a mechanism dependent on TLR4-TRIF signaling but independent of MyD88 (ref. 34).

NLRs constitute a family of cytosolic proteins capable of detecting pathogens in the cytoplasm. NLRs consist of a central nucleotide-binding oligomerization domain (Nod), a C-terminal leucine-rich domain, and an N-terminal effector domain. NLRs include Nod1 and Nod2, which can sense peptidoglycans or peptides derived from their degradation, and the ICE protease-activating factor (IPAF), which can sense intracellular bacteria or bacterial products such as *Salmonella* flagellin[86]. Immunization of mice with ligands specific for Nod1 or Nod2 induces predominantly T$_H$2 responses. However, in conjunction with TLR stimulation, Nod1 and Nod2 are required for the induction of T$_H$1, T$_H$2 and T$_H$17 responses[87,88]. The cellular and molecular mechanisms that mediate the preferential T$_H$2 response through Nod1 or Nod2 signaling remain to be determined. Furthermore, it was recently demonstrated that alum, a classic T$_H$2 adjuvant, signals through the NALP3 inflammasome[89–91]. Thus, DCs or macrophages stimulated *in vitro* with alum plus LPS induce IL-1β and IL-18 in a manner dependent on caspase-1 and NALP3 (refs. 89–91). Despite

the convincing *in vitro* studies, the question of whether NALP3 is required for the adjuvanticity of alum remains controversial, with some studies demonstrating abrogation of antibody responses in *Nalp3*^{−/−} mice[89,91] and other studies showing partial or no effects[90]. However, the mechanisms by which alum induces T_H2 responses are poorly understood.

CLRs belong to a large superfamily of transmembrane and soluble proteins that sense carbohydrate components of several pathogens, as well as self-glycoproteins. Like TLRs, recent studies have shown that CLRs can activate various signaling pathways that can induce DCs to stimulate T_H1, T_H2 or T_H17 cell responses[92]. For example, dectin-1- and dectin-2-mediated signaling programs DCs to produce IL-6 and IL-23 and induce T_H17 cell responses[93,94]. DC-SIGN (DC-specific intercellular adhesion molecule-3-grabbing nonintegrin) is a receptor with a broad pathogen recognition specificity as a result of its affinity for mannose and fucose carbohydrates. Depending on the type of pathogen, DC-SIGN can contribute to either T_H1, T_H2 or T_{reg} cell responses. For example, mycobacteria target DC-SIGN to suppress DC function[95], and the LPS Lewis antigen (Le) of *Helicobacter pylori* induces T_H2-biased responses through a mechanism dependent on DC-SIGN; in contrast, Le[−] variants of *H. pylori* escape binding to DC-SIGN and induce a T_H1 response[95]. Allergens also signal through DC-SIGN to induce T_H2 responses. Thus, the main glycoprotein allergen from peanuts (*Arachis hypogaea*), Ara h 1, is a ligand of DC-SIGN and acts as a T_H2 adjuvant *in vitro*[22].

Antigen-antibody complexes can regulate innate and adaptive immune responses through interaction with Fc receptors, which bind to the Fc region of antibodies[96]. Ligation of Fc receptors that bind IgG antibodies (FcγRs) on macrophages and DCs inhibits the induction of IL-12 by TLRs and enhances that of IL-10, and programs these cells to induce a T_H2-biased response[97,98]. FcγRs can be either activating or inhibitory: in their intracellular domains, FcγRI (CD64) and FcγRIII (CD16) possess immunoreceptor tyrosine-based activation motifs (ITAMs), whereas FcγRIIb possesses immunoreceptor tyrosine-based inhibitory motifs (ITIMs)[96]. The activating FcγR receptors, FcγRI and FcγRIII, signal through a common signaling partner, FcRγ (FcεRIγ), which contains an ITAM motif[96]. FcγRIIb is the only member of the inhibitory Fcγ receptor family in mice, and does not associate with FcRγ. Interestingly, FcγRIII ligation on DCs inhibits TLR4-mediated IL-12 induction by the DCs but enhances IL-10 induction, thus programming the DCs to induce T_H2-biased responses and enhance airway inflammation[99].

Complement activation is one of the earliest innate responses and is critical to host defense as well as to adaptive immunity. Activation of complement receptor 3 (CR3)[100] and CD46 (ref. 101) by C3 cleavage products inhibits TLR-induced IL-12 production in monocytes. Furthermore, complement-derived C5a anaphylatoxin inhibits TLR4- and CD40-induced IL-12, IL-23 and IL-27 production in macrophages by extracellular signal-regulated kinase (Erk)– and phosphatidylinositol-3-OH kinase–dependent pathways, resulting in diminished T_H1 responses[102].

A key feature of several T_H2-inducing stimuli, including allergens and parasites, is that they possess cysteine or serine protease activities[103]. This suggests that protease activity might be key in T_H2 responses induced by such allergens and parasites. Indeed, when these proteases are administered *in vivo*, they produce T_H2 responses[103]. Thus, the protease activities of house dust mite, *Aspergillus*, ragweed and papain are essential for the induction of T_H2 responses. However, the cellular and molecular mechanisms by which proteases are sensed by the immune system, and how they induce T_H2 responses, are not understood. Although protease-activated receptors (PARs) are

activated by serine proteases, the ability of PARs to recognize cysteine protease activity and mediate T_H2 responses is poorly understood. Recently, PAR-2 was shown to mediate, in part, the induction of TSLP from airway epithelial cells *in vitro* in response to the protease activity of the common environmental fungus *Alternaria alternata*, as well as in response to the cysteine protease papain[104]. Moreover, the serine protease kallikrein-5 induces atopic dermatitis–like lesions through PAR-2-mediated induction of TSLP in Netherton syndrome[105]. Netherton syndrome is a severe skin disease with persistent atopic manifestations, caused by mutations in the gene encoding serine protease inhibitor Kazal-type-5 (SPINK5, also known as LEKTI)[105]. This protease inhibitor mutation results in unregulated kallikrein-5 activation of PAR-2, with the induction of the pro-T_H2 mediators TSLP, TARC and MDC[105].

Hierarchy level −2: signaling networks

Certain TLR2 ligands and SEA induce an enhanced duration and magnitude of Erk signaling in DCs, compared to that induced by other TLR ligands[69,70]. Interestingly, DCs from *Erk1*^{−/−} mice, or human DCs treated with a synthetic inhibitor of MEK1 and MEK2 (upstream activators of Erk1 and Erk2), produce enhanced amounts of IL-12p70 and diminished amounts of IL-10 in response to TLR stimulation[69,70], consistent with previous reports that Erk suppresses the induction of IL-12 and enhances IL-10 induction[106]. Sustained duration and magnitude of Erk signaling results in phosphorylation and stabilization of the early growth transcription factor c-Fos in fibroblasts. Consistent with this, phosphorylated c-Fos expression is enhanced in DCs stimulated by TLR2 ligands and SEA, relative to that in DCs stimulated with *E. coli* LPS or flagellin[69,70]. Furthermore, DCs from *Fos*^{−/−} mice, or human DCs in which c-Fos is knocked down with short interfering RNA, produce more IL-12p70 in response to TLR2 stimulation[69,70]. Consist with this, bacterial teichoic acids can suppress IL-12 induction by certain strains of *Lactobacillus*, and enhance IL-10 production, by means of TLR2-dependent Erk signaling[107]. In addition to TLR2 ligands, a diverse range of stimuli, such as cyclic AMP[108] and the complement proteins C5a[102] and iC3b[109], cigarette smoke–induced oxidative stress[110], and signaling through DC-SIGN[111], FcγR[112], the human osteoclast-associated receptor (OSCAR, an FcγR-associated receptor[113]) and cannabinoid CB2 receptor[114], can negatively regulate IL-12 production and, in some cases, promote IL-10 production, through a mechanism dependent on Erk signaling. In at least the case of cAMP, this was shown to be dependent on c-Fos[108] (**Fig. 2a**).

Further studies have established a key role for mitogen-activated protein-3 kinase (also known as Tpl2) in TLR-mediated activation of Erk in macrophages, DCs and B cells[115,116]. Furthermore, abrogation of Erk signaling in *Tpl2*^{−/−} macrophages reduces c-Fos expression and transcriptional activity[116]. Interestingly, Tpl2 amounts are low in *Nfkb1*^{−/−} mice; however, rescue of TLR-dependent Erk activation in *Nfkb1*^{−/−} in bone marrow–derived macrophages only partially restores IL-10 production, suggesting both Erk-independent and Erk-dependent mechanisms of IL-10 production[115]. Collectively, these findings indicate that the Erk-Fos signaling pathway is an important regulator of IL-12 production in DCs and macrophages.

A second signaling pathway that programs DCs to induce T_H2 responses is one that involves TSLP, which stimulates NF-κB (predominantly the p50 subunit–containing form) in DCs to induce OX40L, which facilitates T_H2 differentiation (**Fig. 2b**). TSLP signaling also induces the activation of STAT6, which programs DCs to secrete chemokines necessary for the recruitment of T_H2 cells. In addition, TSLP signaling limits the activation of STAT4 and interferon
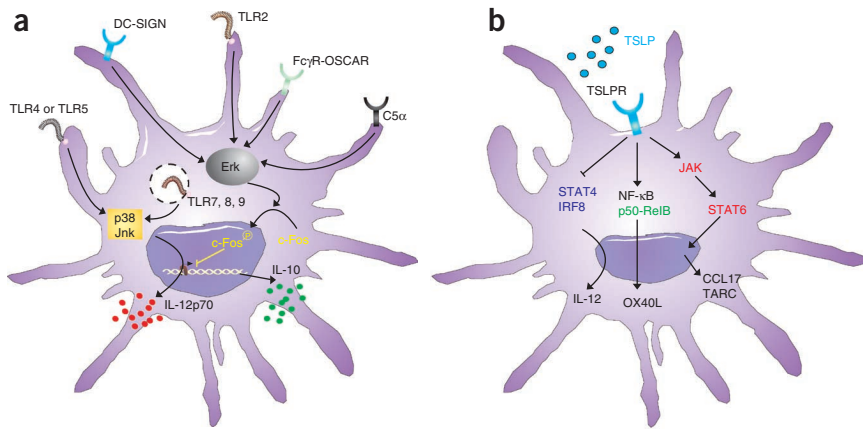
Figure 2 Signaling pathways that inhibit IL-12 production and program DCs to induce T$_H$2 responses. (**a**) Signaling through TLRs 4, 5, 7, 8 and 9 induces robust activation of the MAP kinases p38 and Jnk1/2, which leads to the induction of IL-12 and T$_H$1 responses. In contrast, signaling through TLR2, DC-SIGN, OSCAR (an FcγR-associated receptor) and C5a induces enhanced and sustained activation of Erk1 and Erk2, which results in the stabilization of the transcription factor c-Fos that suppresses IL-12 and enhances IL-10, thus favoring a T$_H$2 bias. (**b**) TSLP receptor (TSLPR) signaling pathways program DCs to induce potent T$_H$2 responses. Signaling through TSLPR in DCs leads to activation of the transcription factors NF-κB and STAT6, which are critical for promoting T$_H$2 responses. Activation of NF-κB (predominantly p50) induces expression of OX40L, which stimulates T$_H$2 differentiation. Furthermore, activation of STAT6 triggers DCs to secrete chemokines necessary for the recruitment of T$_H$2 cells. In contrast, TSLPR signaling inhibits activation of STAT4 and IRF8, critical for the production of the T$_H$1-polarizing cytokine IL-12.

regulatory factor-8 (IRF-8), essential factors for the production of the T$_H$1-polarizing cytokine IL-12. By contrast, TLR ligands and CD40 ligand do not activate STAT6 in myeloid DCs, but instead increase the abundance of STAT4 and IRF-8 to induce T$_H$1 responses through the production of IL-12 (ref. 117).

Third, our recent findings demonstrate a distinct program of DC activation by papain. DCs cultured with papain do not produce any pro- or anti-inflammatory cytokines or chemokines, among more than 20 tested. Instead, there is an induction of genes encoding several reactive oxygen species (ROS)-related molecules, including heme oxygenase (decycling)-1 (HO-1) and NCF4(P40phox)[34]. HO-1 is recognized as a sensitive and reliable indicator of cellular oxidative stress, and NCF4(p40phox) is a subunit of NADPH complex[118]. The production of ROS by DCs was confirmed by staining

with the 5-(and-6)-carboxy-2′,7′-dichloro-dihydrofluorescein diacetate (DCF) dye[34]. Presence of ROS is an endogenous signal for induction of inflammation, acute lung injury and artherosclerosis[119,120]. Although the role of ROS in asthma is well documented[121], involvement of ROS in induction of T$_H$2 responses to cysteine proteases (helminth products and allergens) is as yet unknown. It has been reported that production of ROS by macrophages diminishes a T$_H$1 response[122]. Consistent with this, inhibition of ROS in papain-treated DCs results in enhanced IL-12 and CD70 production and enhanced T$_H$1 responses. Furthermore, targeting synthetic inhibitors of ROS to DCs *in vivo* using nanoparticles impairs papain-induced T$_H$2 responses. Thus, papain induction of ROS in DCs programs them to suppress T$_H$1 and promote T$_H$2 responses.

In the case of tolerogenic responses, yeast zymosan, which signals through dectin-1 and the dimer of TLR2 and TLR6, induces DCs to express IL-10 and the retinoic acid–metabolizing enzyme retinaldehyde dehydrogenase type 2 (RALDH2), by means of an Erk-dependent pathway[75,76]. Retinoic acid induces SOCS3 expression in DCs in an autocrine manner, which suppresses activation of p38 MAPK and proinflammatory cytokines[76] (**Fig. 3a**). Consistent with this, TLR2 signaling induces development of T$_{reg}$ cells and suppresses IL-23-, T$_H$1- and T$_H$17-mediated auto-immune responses *in vivo*. Similarly, activation of TLR2 by LcrV or *Lactobacillus* induces Erk-dependent suppression of IL-12 and induction of IL-10 (refs. 58,107). Thus, TLR2-Erk dependent induction of IL-10 and RALDH enzymes programs tolerogenic DCs.

Disruption of E-cadherin–E-cadherin interactions between DCs promotes 'alternative' maturation of immature DCs, with impaired immune stimulatory capacity[49]. However, the mechanism by which β-catenin signaling programs DCs to a tolerogenic state is not known. Our recent work shows that, unlike in splenic DCs, β-catenin signaling is constitutively active in intestinal DCs and macrophages[123].

Figure 3 Signaling pathways that program DCs to induce tolerogenic responses. (**a**) TLR-Erk-mediated induction of vitamin A metabolizing enzymes and IL-10. Triggering DCs through TLR2-TLR6 leads to Erk activation, which mediates induction of retinaldehyde dehydrogenase-2 (RALDH2). This results in the conversion of retinal to retinoic acid (RA), which then exerts an autocrine effect on DCs by means of the receptors RAR or RXR to induce SOCS3, which suppresses activation of p38 MAPK and proinflammatory cytokines. Further, IL-10 and RA program DCs to induce T$_{reg}$ cells and limit inflammatory responses. (**b**) β-catenin signaling pathways in programming regulatory DCs. Activation of β-catenin pathway by E-cadherin, TLRs or Wnt ligands in DCs promotes induction of anti-inflammatory factors such vitamin A, IL-10 and TGF-β that are critical for promoting T regulatory response and limiting inflammatory responses. Fzd, the Wnt receptor Frizzled.
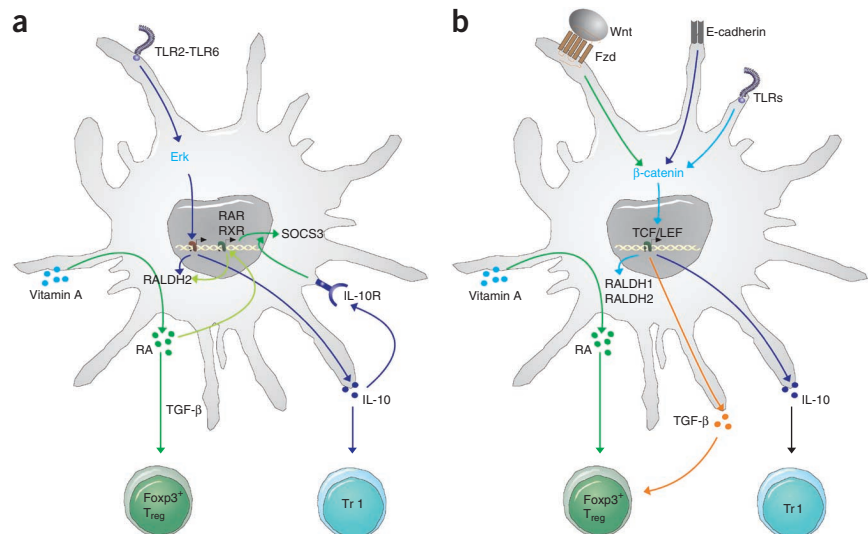
DC-specific deletion of the gene encoding β-catenin in mice leads to markedly reduced frequencies of Foxp3^{+} regulatory T cells, and enhanced frequencies of T_H1 and T_H17 cells, in the intestine but not in the spleen[123]. Consistent with this, intestinal DCs deficient in β-catenin show reduced expression of RALDH enzymes and IL-10 production and promote inflammatory T cells responses in the steady state[123]. Collectively, these data illustrate the emerging role of β-catenin signaling in programming DCs to promote intestinal homeostasis and tolerance (**Fig. 3b**).

Hierarchy level +1: cell-cell cooperation

Several cell types interact to shape T_H2 and tolerogenic responses. For example, basophils produce IL-4 and promote T_H2 responses[35–43]. Recent studies show that basophils can present antigens to CD4^{+} T cells during T_H2 responses[41–43]. However, our recent work demonstrates that DCs are far more efficient than basophils at presenting protein antigens and inducing proliferation of naive CD4^{+} T cells[35]. When lymph node DCs and basophils are isolated after immunization of mice with ovalbumin plus papain, and cultured with naive ovalbumin-specific CD4^{+} T cells, coculture of 5×10^3 to 10^4 DCs with 10^5 T cells is sufficient to induce robust T cell proliferation (in the absence of exogenous antigen), but is unable to induce T_H2 polarization[34]. In contrast, coculture of 5×10^3 to 10^4 basophils with 10^5 T cells does not induce significant T cell proliferation, but does result in IL-4 secretion *in vitro*. Notably, coculture of DCs, basophils and T cells results in robust T cell proliferation and T_H2 induction[34]. Consistent with this, deletion of DCs *in vivo* using the CD11-DTR mice, before immunization with papain plus ovalbumin, results in impaired ovalbumin-specific T cell proliferation and T_H2 responses. In contrast, deletion of basophils results in impaired T_H2 induction but normal proliferation. Thus, induction of T_H2 responses to papain is dependent on both DCs and basophils[34].

Further evidence for intercellular cooperation comes from three research groups who have identified new populations of cells involved T_H2 responses[124–126]. First, a subset of lymphoid cells in fat-associated lymphoid tissues (FALC) has been identified in both mice and humans[124]. These cells are lineage marker–negative (Lin[−]) but Sca-1^{+}c-Kit[+]IL-7R^{+}IL-33R^{+}. Such cells proliferate in response to IL-2 and produce copious IL-5, IL-6 and IL-13. Consistent with the effects of IL-5 and IL-6 on B cell differentiation, these cells support the self-renewal of B1 B cells and enhance IgA production. After helminth infection and in response to IL-33, FALC Lin[−]c-Kit[+]Sca-1^{+} cells produce abundant IL-13, which leads to goblet cell hyperplasia—a critical step for helminth expulsion. Second, a new innate effector leukocyte ('nuocyte')[126] has been identified that expands *in vivo* in response to the T_H2-inducing cytokines IL-25 and IL-33, and is the chief producer of IL-13 during infection with the helminth *N. brasiliensis*. Nuocytes express ICOS, ST2, IL-17RB and IL-7Rα, although only a subset of them express c-Kit. In the absence of IL-25 and IL-33 signaling, nuocytes fail to expand, resulting in impaired worm expulsion[126]. Finally, IL-25 promotes the accumulation in the gut-associated lymphoid tissue of a Lin[−]Sca-1^{+}c-Kit[int] multipotent progenitor population (MPP type2) that has the capacity to give rise to monocytes or macrophages and to granulocytes. Transfer of MPP type2 cells rescues T_H2 responses and immunity to *T. muris* in $Il25^{-/-}$ mice. The potential relationships between these cells, and their relative contributions to T_H2 induction in a variety of settings, needs to be studied. Finally, in the case of tolerogenic responses, there are many examples of cell-cell cooperation. Thus, myeloid-derived suppressor cells are a heterogenous mix of cells that expand during cancer, inflammation and infection and potently suppress T cell responses. These cells may augment the immunosuppressive effects of tolerogenic DCs[127].

Hierarchy level +2: tissue microenvironments

This topic is discussed extensively in another review in this focus issue[128], and will thus be discussed only briefly. For example, intestinal epithelial cells secrete IL-25 and TSLP and condition intestinal DCs to express RALDH enzymes, which programs them to induce T_{reg} cells. In addition to epithelial cells, stromal cells are also critical in conditioning DCs to regulatory or tolerogenic states in various organs, such as the gut-associated lymphoid tissue and spleen[128]. Furthermore, allergens trigger the production of TSLP, GM-CSF and IL-33 by airway epithelial cells, which induce DCs to migrate to the lymph nodes and prime T_H2 responses[128].

Top-down, bottom-up or middle-out?

The temporal and spatial complexity of the events that lead to T_H2 or tolerogenic responses can be simplified by considering different levels of abstraction, or 'hierarchies of organization'. Many important insights have emerged from studies that focus on a single level of the hierarchy (for example, signaling pathways in DCs, or cellular interactions in the lymph nodes), but such insights do not offer a global picture. Therefore, future research should seek an integrated understanding of the immune response at several levels: signaling within DCs, nature of the innate receptors, cell-cell interactions and the influence of microenvironments. A key question is whether investigations should occur in a 'top-down' (for example, hierarchy levels +2 to −2), or 'bottom-up' direction. In fact, given their central role in orchestrating the response, it may be most sensible to consider DCs as a node from which investigations could occur in a 'middle-out' manner. It is clear that a unified theory of T_H2 or T_{reg} responses will only be possible by integrating information obtained at each of these hierarchical levels. Such understanding will guide the rational design of therapeutics and vaccines that can reprogram the innate immune systems toward tolerance in active T_H2- or T_H1-mediated disease.

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