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Toll-like receptor control of the adaptive immune responses

Akiko Iwasaki & Ruslan Medzhitov

Recognition of microbial infection and initiation of host defense responses is controlled by multiple mechanisms. Toll-like receptors (TLRs) have recently emerged as a key component of the innate immune system that detect microbial infection and trigger antimicrobial host defense responses. TLRs activate multiple steps in the inflammatory reactions that help to eliminate the invading pathogens and coordinate systemic defenses. In addition, TLRs control multiple dendritic cell functions and activate signals that are critically involved in the initiation of adaptive immune responses. Recent studies have provided important clues about the mechanisms of TLR-mediated control of adaptive immunity orchestrated by dendritic cell populations in distinct anatomical locations.

Sensing and defeating microbial infections is essential to the survival of metazoan species. The reliable detection of pathogens is a daunting task because of their molecular heterogeneity and rapid evolution. Several recognition strategies have evolved to deal with this problem. The pattern-recognition strategy is based on the detection of a limited set of conserved molecular patterns that are unique to the microbial world and invariant among entire classes of pathogens[1]. The targets of pattern recognition (sometime called pathogen-associated molecular patterns (PAMPs)) are detected by pattern recognition receptors (PRRs) that signal to the host the presence of infection. The 'missing self' strategy is based on the detection of molecular features (host gene products, host-specific protein or lipid modifications) that are unique to the host and absent from the pathogens or any other foreign entity. In this case, recognition of these molecular structures is coupled with inhibitory pathways, such as those used by the complement and the natural killer (NK) cell systems, in which the lack of recognition is associated with the induction of one of several effector responses, including phagocytosis or complement- or NK cell–mediated cell lysis. Although pattern recognition is universally used in the animal kingdom, it seems to have only a limited, if any, function in plant immunity. It has been proposed that instead of detecting the pathogen-specific molecules, plants may have evolved a recognition strategy that detects pathogen-specific activities[2]. In this case, dedicated gene products (referred to as resistance gene products) specialize in sensing 'suspicious activities' or intracellular processes that are universally associated with microbial infections. Because animal cells use a very similar strategy to detect various types of cellular stresses, it is likely that such a strategy may also be used for the detection of intracellular infections in animals.

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The Toll-like receptor (TLR) family is the best characterized class of PRRs in mammalian species. Although the exact gene numbers may differ between species, it is likely that most mammalian species have 10 to 15 TLRs. TLRs detect multiple PAMPs[3], including lipopolysaccharide (LPS) (detected by TLR4), bacterial lipoproteins and lipoteichoic acids (detected by TLR2), flagellin (detected by TLR5), the unmethylated CpG DNA of bacteria and viruses (detected by TLR9), double-stranded RNA (detected by TLR3) and single-stranded viral RNA (detected by TLR7)[4–6]. TLRs 1, 2, 4, 5 and 6 seem to specialize in the recognition of mainly bacterial products that are unique to bacteria and not made by the host. Their detection therefore affords a straightforward self–non-self discrimination. TLRs 3, 7, 8 and 9, in contrast, specialize in viral detection and recognize nucleic acids, which are not unique to the microbial world. In this case, self–non-self discrimination is mediated not so much by the molecular nature of the ligands as by their accessibility to the TLRs. These TLRs are localized to intracellular compartments[7–9] and detect viral nucleic acids in late endosomes-lysosomes[5,6,10]. Because the host's nucleic acids are not normally accessible in these compartments, they do not trigger TLRs. However, in certain conditions, such as deficient clearance of apoptotic cells, the host-derived nucleic acids (often in complex with DNA- or RNA-binding proteins) may become available for activating TLRs, which may break tolerance and lead to autoimmunity[11].

In addition to ligand specificity, the functions of individual TLRs differ in their expression patterns and the signal transduction pathways they activate. The biological importance of a given combination of the expression profile, ligand specificity and signaling specificity for each individual TLR is unknown. These differences notwithstanding, all TLRs sense microbial infection and trigger a multitude of antimicrobial and inflammatory responses. In addition, a subset of TLR-induced signals is dedicated to the control of adaptive immunity. We will discuss next recent advances in our understanding of the mechanism of the TLR-mediated control of adaptive immune responses.

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TLR-regulated cell recruitment to infection sites

One of the distinctive features of the immune system is that it relies on cell migration for surveillance, attack, containment and clearance of invading pathogens. Two types of cell migration are used by the cells of the innate and adaptive immune systems: inducible and homeostatic. Inducible cell migration is generally triggered as a result of the sensing of pathogens through PRRs, as in neutrophil recruitment to the local site of infection. Steady-state, or homeostatic, cell migration allows naive lymphocytes to circulate between the secondary lymphoid tissues throughout the body to enhance their chances of encountering the cognate antigen. This pathway is constitutive and does not require induction through TLRs. In this section, inducible cell migration strategies used by the innate immune system will be discussed.

Innate recognition of PAMPs through TLRs initiates an inflammatory response characterized by the recruitment of cells to the sites of infection to augment the killing of invading pathogens and to halt their spread. Cell migration from peripheral blood into the inflamed tissue involves a tightly controlled sequence of events[12]. These events are mediated by two types of signals: diffusible chemotactic factors and cell surface adhesion molecules. Activation of TLRs induces the expression of selectin, chemokine and chemokine receptor genes that regulate cell migration to the sites of inflammation[13]. Sensing inflammatory signals, leukocytes first roll on the vascular endothelial cells, a process mediated by selectins expressed by the endothelium and the carbohydrate ligands on leukocytes. The selectins are rapidly displayed on the endothelial cell surface either after direct recognition of pathogens through TLRs or by tumor necrosis factor secreted from activated tissue macrophages. Next, PAMP recognition through the TLR induces chemokine secretion. Key inflammatory chemokines produced during acute microbial infection include interleukin 8 (IL-8, also known as CXCL8), growth-related oncogene-α (GRO-α; CXCL1), monocyte chemoattractant protein 1 (MCP-1, also known as CCL2), MCP-2 (CCL8), MCP-3 (CCL7), MCP-4 (CCL13), macrophage inflammatory protein 1α (MIP-1α, also known as CCL3), MIP-1β (CCL4) and RANTES (CCL5)[14]. These chemokines bind to the luminal surface of the vascular endothelium and trigger activation of the leukocytes, inducing conformational changes in the integrins[12]. The activated integrins on the leukocytes allow firm adhesion to the vascular endothelium. Integrin ligands on the endothelium, such as intercellular cell adhesion molecule1, are also induced either directly by TLRs expressed on the endothelium, or indirectly, through tumor necrosis factor and IL-1, which are induced by TLR- activated resident macrophages. Finally, the adherent leukocytes migrate between the endothelial cells and extravasate into tissue interstitium.

To provide maximum surveillance for infectious agents, in addition to the inflammation-induced cell recruitment, most tissues of the body, particularly at the mucosal surfaces that represent portals of pathogen entry, are interlaced with resident innate leukocytes such as dendritic cells (DCs), macrophages and mast cells. Pathogen recognition through TLRs orchestrates the recruitment of leukocytes to the sites of infection by activating the tissue stromal cells, tissue-resident innate cells and the circulating leukocytes.

TLR activation of leukocytes

Acute inflammatory cellular infiltrate consists of innate immune cells such as monocytes, neutrophils, basophils, eosinophils and NK cells. Neutrophils and NK cells are critical effector cells that protect the host by killing pathogenic microbes and infected cells, respectively. Studies examining TLR expression on innate leukocytes and other cell types have been complicated by the discrepancy between mRNA expression and responsiveness to the TLR agonists, lack of reliable antibodies to TLRs and some species-specific differences in TLR expression. In addition, expression of some TLRs is regulated, both positively and negatively, in several cell types. Nevertheless, analysis of TLR expression at the mRNA level has provided some valuable information. Human neutrophils express mRNA for TLR1 through TLR10, except for TLR3, and secrete inflammatory chemokines after stimulation with LPS, zymosan and R848 (ref. 15). However, neutrophils are not stimulated by CpG[16]. Similarly, NK cells express TLR9 mRNA, but are not responsive to CpG stimulation[17]. The potent ability of CpG DNA to stimulate NK cells was attributed to the contaminating plasmacytoid DCs (pDCs), which, after being stimulated with CpG, secrete large amounts of type I interferons and induce NK cell activation[17]. The activated NK cells secrete interferon-γ (IFN-γ) and augment pathogen clearance by macrophages. Eosinophils, despite their constitutive expression of TLR1, TLR4, TLR7, TLR9 and TLR10 mRNA, only respond to stimulation with R848 through TLR7, which results in their activation and prolonged survival[18]. Mast cells express TLRs 1, 2, 4 and 6 and respond to TLR2 and TLR4 agonists[19–21]. Monocytes express a variety of TLRs that induce their activation (discussed below). The monocytes that infiltrate the inflamed tissue can differentiate into either tissue macrophages or DCs that contribute to the generation of adaptive immunity. TLR signals not only regulate chemokine and chemokine receptor gene expression but also control cell surface chemokine receptor expression by inhibiting chemokine receptor desensitization. Neutrophils stimulated through TLR4 transcriptionally downregulate the expression of G protein–coupled receptor kinases, allowing these cells to be responsive to chemokines for a prolonged period of time[22].

TLR activation of stromal cells

The epithelial layer provides the first line of defense against invading pathogens. Consequently, many TLRs are expressed on the epithelial cells of the intestinal, respiratory and urogenital tracts[23,24]. Pathogen recognition by the TLRs expressed by these cells leads to the production of cytokines, chemokines and antimicrobial peptides[25,26]. The epithelial chemokines can be displayed on the luminal surface of both the local tissue and draining lymph nodes vascular endothelium and participate in cell recruitment. Cell migration is also regulated by direct recognition of PAMPs by the vascular endothelium. In a mouse model of septic shock, systemic LPS injection induces rapid neutrophil sequestration into the lungs. In one study of bone marrow–chimeric mice that were TLR4-deficient in either their leukocytes or in the stromal cell compartment TLR4 responsiveness by the vascular endothelial cells, but not neutrophils, was required for migration of these cells into the lung tissue[27]. In another study, TLR4 expression in both the hematopoietic and the stromal compartments was required for the recruitment of leukocytes after challenge with the uropathogenic *Escherichia coli*[28]. Thus, the recognition of PAMPs by epithelial, endothelial and hematopoietic cells through TLRs are integral in the innate immune defense against microbial pathogens at sites of infection. Future studies will need to address the cell type–specific functions of TLRs in stroma and hematopoietic cells, as well as in different classes of leukocytes.

TLRs and DCs

DCs are central to T lymphocyte activation and differentiation into T helper type1 (T_H1) cells, T_H2 cells and cytotoxic T lymphocyte (CTL) effectors[29]. DCs take up antigens, become activated and migrate to

Table 1 TLR expression by human DC subsets

	Freshly isolated			*In vitro*–differentiated DCs
	Monocyte	mDC	pDC	GM-CSF + IL-4
TLR1	++	++	+	++
TLR2	++	++	–	++
TLR3	–	++	–	++
TLR4	++	–	–	++
TLR5	++	+	–	+[37] –[32,36]
TLR6	++	++	++	++
TLR7	+[34,35] –[17,32,33,36,37]	+[34,35] –[32,33]	++	–
TLR8	++	++	–	++
TLR9	–	–	++	–
TLR10	–	+	+	
Refs.[a]	17,32–37	32–35	9,17,32–35	9,32,36,37

+, ++ and – indicate the relative mRNA expression of each TLR by the CD subsets. References superscripted to the right of + or – indicate conflicting results: some studies found expression (+), whereas others found no expression (–), of a given TLR on a particular cell type. GM-CSF, granulocyte-macrophage colony-stimulating factor.
[a]These references were used to determine the TLR expression pattern of the specific cell type listed in each column.

local lymphoid tissues to present the antigenic peptides on the relevant major histocompatibility complex (MHC) molecules. This process involves phagocytosis, the upregulation of costimulatory and MHC molecules, a switch in chemokine receptor expression, the secretion of cytokines and chemokines and the presentation of antigens by DCs[29]. All of these events are regulated through the recognition of pathogens via the PRRs expressed by DCs and are influenced by the microenvironment in which they reside. Many types of PRRs are expressed by DCs, including C-type lectins, mannose receptors and TLRs. However, TLR family members represent critical PRRs, whose signals lead to the generation of effector responses including T_H1 (ref. 30) and CTL[31] responses. In both mouse and human, distinct DC subsets occupy special niches defined by their anatomical location and their ability to respond to certain types of pathogens through the expression of distinct sets of TLRs.

TLR expression on DC subsets

Studies of DC subsets isolated from humans and mice have revealed that TLRs have distinct expression patterns (**Tables 1** and **2**). Freshly isolated human pDCs express TLR7 and TLR9, whereas $CD11c^+$ human myeloid DCs (mDCs) express TLR1, TLR2, TLR3, TLR5, TLR6 and TLR8 (refs. 17,32,33; **Fig. 1**). In some studies, TLR7 expression was detected on both pDCs and mDCs[34,35], whereas others found TLR7 was exclusively expressed in pDCs[32,33]. Human blood monocytes express TLRs 1, 2, 4 and 5, but progressively lose these receptors as they differentiate into immature DCs in the presence of granulocyte-macrophage colony-stimulating factor and IL-4. These cells instead acquire the expression of TLR3[36]. Notably, human mDCs and *in vitro*–differentiated immature DCs express TLR3 in their intracellular compartments[9], unlike human fibroblasts, which express TLR3 on the cell surface. Human Langerhans cells do not express TLR3 (ref. 37).

In mice, all splenic DC subsets express TLRs 1, 2, 4, 6, 8 and 9 (ref. 38). However, mouse pDCs do not express TLR3. Moreover, mouse $CD8\alpha^+$ DCs lack TLR5 and TLR7 expression and fail to respond to TLR7 agonists[38,39] (**Fig. 1**). There are also distinct differences in the expression and responsiveness of certain TLRs in freshly isolated DCs versus *in vitro*–derived DCs. Although TLR4 is expressed at low amounts on splenic DCs, freshly isolated mouse DC subsets do not respond to LPS stimulation[40]. In contrast, $CD11c^+CD11b^+$ DCs derived from bone marrow precursors in the presence of granulocyte-macrophage colony-stimulating factor have high expression of TLR4 and respond robustly to LPS[40], a characteristic shared with murine macrophages.

With the exception of a few studies[9,41,42], most of the work examining the TLR expression profiles on DC subsets used RT-PCR because of the lack of antibodies available to detect individual TLRs. However, inhibitors of endosomal pathways such as chloroquine and bafilomycin have been used to delineate the functional location of certain TLRs within the DC subsets. These studies showed that TLR3, TLR7, TLR8 and TLR9 all require an acidic environment for activation of the endosomes, whereas TLRs 1, 2, 4, 5, and 6 are expressed on the DC cell surface and do not require endosomal maturation[7–9,42].

The TLR expression profiles of distinct DC subsets raise some interesting questions about the function of these DCs. For example, TLR3 is lacking in both human and mouse pDC populations. Yet pDCs are very efficient detectors of viral infections, recognizing the viral genomes through TLR9 (double-stranded DNA)[10,43] and TLR7 (single-stranded RNA)[5–7]. Another point that arises is the lack of responsiveness of both human and mouse DC subsets to LPS. Only monocytes and *in vitro*–differentiated DCs express TLR4 and respond notably to LPS stimulation. These data suggest that some, if not all, of the immunostimulatory effects induced by injected LPS on DCs are perhaps mediated indirectly by non-DC or DC precursors *in vivo*. Whichever is the case, the accumulating evidence points to a differential function for DC subsets in pathogen recognition and influencing adaptive immune responses, which will be discussed in detail in the following sections.

TLR-dependent DC-mediated control of T cell activation

The generation of adaptive immunity begins with DCs capturing microbial antigens in the peripheral tissues. Subsequently, DCs

Table 2 TLR expression by mouse DC subsets

	Freshly isolated DCs				*In vitro*–differentiated DCs
	$CD4^+$	$CD8^+$	DN	pDC	GM-CSF + IL-4
TLR1	++	++	++	++	
TLR2	++	++	++	++	
TLR3	–	++	++	–	
TLR4	++[38] –[40]	++[38] –[40]	++[38] –[40]	++[38] –[40]	++
TLR5	++	–	++	+	
TLR6	++	++	++	+	
TLR7	++	–	++	++	
TLR8	++	++	++	++	
TLR9	++	++	++	++	++
Refs.[a]	38	38	38	38	40

+, ++ and – indicate the relative mRNA expression of each TLR by the CD subsets. References superscripted to the right of + or – indicate conflicting results: some studies found expression (+), whereas others found no expression (–), of a given TLR on a particular cell type.
[a]These references were used to determine the TLR expression pattern of the specific cell type listed in each column.

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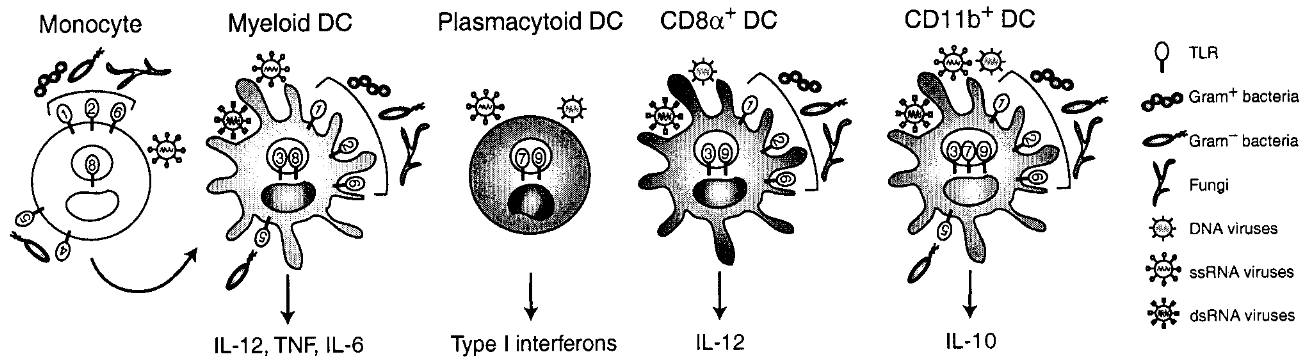

Figure 1 DC populations express nonoverlapping sets of TLRs. Human peripheral blood myeloid DCs and monocytes express distinct sets of TLRs on their cell surfaces and in the lysosomal compartment. Myeloid DCs express a variety of surface TLRs and can recognize bacterial, fungal and viral pathogens and secrete the inflammatory cytokines IL-12, tumor necrosis factor (TNF) and IL-6. Although they have a similar set of TLRs, monocytes do not express TLR3 but upregulate TLR3 as they mature into DCs. Both human and mouse pDCs express TLR7 and TLR9, respond to viruses and secrete type I interferons. In the secondary lymphoid organs, blood-derived DC precursors give rise to DCs. In mice, these include $CD11b^+$ and $CD8\alpha^+$ DCs that express mostly overlapping, but not identical, sets of TLRs. Less is know about the lymphoid organ resident blood-derived DC subsets in humans. ss, single-stranded; ds, double-stranded. Numbers on cells indicate the TLR expressed by each DC subset.

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migrate to the draining lymph nodes to present the processed peptides to naive T lymphocytes in the context of MHC molecules. This migration is mediated by TLR-induced downregulation of inflammatory chemokine receptors and upregulation of the receptors for lymphoid chemokines. The stimulation of immature DCs with TLR ligands results in CCR6 downregulation and CCR7 upregulation[44,45], which enhances the ability of DCs to migrate from the peripheral tissues to the draining lymph node[46,47]. In transit, DCs also undergo a maturation program that endows the cells with the ability to stimulate naive T lymphocytes. It is only after encountering microbial pathogens that DCs begin the process of maturation.

Once inside the lymph nodes, DCs migrate to the T cell areas, seek out antigen-specific T cells and induce their activation and differentiation into effector cells. DCs provide the naive T cell with two signals required for their activation. The first signal is the antigen-specific signal received as a result of binding of the T cell receptor to peptide presented by the MHC molecule. The second signal is provided by costimulatory molecules such as B7-1 (CD80) and B7-2 (CD86), which are expressed by the DCs and which trigger CD28 expressed on naive T cells. Depending on the density of the peptides presented, types of costimulatory molecules expressed and cytokines secreted by the DCs, naive $CD4^+$ T cells differentiate into either T_H1 or T_H2 cells[48]. Primary activation of $CD8^+$ T cells requires similar signals from the DCs, whereas memory $CD8^+$ T cell responses require $CD4^+$ T cell help[49–52]. The main pathway by which DCs become activated and mature to provide the second signal to naive T cells occurs via the TLR recognition of PAMPs[53].

A given DC population will respond only to the pathogens for which they have appropriate TLRs (**Fig. 1**). Furthermore, stimulation through a given TLR, depending on which DCs express it, can result in a distinct outcome, even when triggered by the same ligand. Stimulation of human pDCs and mDCs with synthetic TLR7 agonists induces the secretion of IFN-α and IL-12, respectively[35]. Similarly, stimulation with certain synthetic oligonucleotides containing CpG motifs induces pDCs to secrete IFN-α and mDCs to secrete IL-12 (ref. 54). Despite these differences in cytokine secretion by DC subsets, signals through TLRs generally result in the activation and maturation of all DCs, as measured by enhanced expression of the costimulatory molecules CD80, CD86 and CD40.

Induction of the costimulatory molecules CD80 and CD86 on the DC surface is a particularly important step in the initiation of adaptive immunity. Indeed it is the coupling of microbial recognition with the induction of the costimulators that allows activation of pathogen-specific T cells, as was originally proposed by Janeway[1]. CD80 and CD86 costimulators 'flag' the antigenic peptide presented by MHC molecules on the same DCs as being microbe derived. Because T cells can receive only the costimulatory signal in an antigen-specific, cognate interaction with DCs, TLR-induced expression of costimulators translates the nonclonal pattern recognition signal into clonal antigen–specific immune responses. It remains unknown, however, how DCs distinguish between antigens derived from self proteins and microbial proteins, as it would be expected that both sources would be available for presentation during infection.

In addition to the costimulatory signals, peripheral tolerance is controlled by regulatory T cells (T_R cells). There are at least two types of these cells: inducible and developmentally programmed. The programmed T_R cells are typically characterized by the expression of CD4 and CD25 molecules and their development and function depends on the transcription factor Foxp3 (refs. 55–57). The inducible T_R cells are not clearly defined in terms of their phenotype or function but are thought to control excessive inflammation by producing large amounts of IL-10 and transforming growth factor-β. The main function of $CD4^+CD25^+$ T_R cells is likely to be the maintenance of tolerance to self antigens. In contrast, these cells should not interfere with the induction of pathogen-specific protective immune responses. One mechanism that allows the activation of pathogen-specific T cells in the presence of T_R cells is a TLR-mediated block of suppression. This mechanism is independent of costimulation, and is instead mediated by IL-6 produced by DCs in response to TLR activation[58]. IL-6, in turn, affects antigen-specific T cells and makes them refractory to suppression by T_R cells. *In vivo*, the release of T_R cell–mediated suppression requires TLR activation on DCs, and this TLR-dependent signal cannot be substituted by other modes of DC activation, such as inflammatory cytokines[59] (C. Pasare and R.M., unpublished observations).

Once $CD4^+$ T cells are activated, their differentiation into effector cells is controlled by a variety of factors, including cytokines produced by DCs. TLR-induced cytokines, such as IL-12, generally spe-

cialize in the induction of T_H1 responses[60]. Accordingly, the immunization of mice with adjuvants containing various TLR ligands, including LPS, CpG DNA and complete Freund's adjuvant, results in T_H1 responses and the induction of T_H1-dependent antibody isotypes. Mice deficient in MyD88, in contrast, are severely compromised in T_H1 differentiation and instead generate T_H2 responses[30,61]. All these data are consistent with a view that TLRs control the induction of T_H1, but not T_H2, responses. However, several reports have demonstrated that triggering TLR2 may induce T_H2 differentiation[62–64]. The physiological importance of this is unclear because pathogens that trigger TLR2, such as Gram-positive bacteria, do not generally induce T_H2 responses *in vivo*. Furthermore, stimulation of lung DCs with low doses of inhaled LPS induces T_H2 responses and allergic inflammation, whereas high dose of LPS in the same conditions induce T_H1 responses[65]. Physiologically, the low dose of LPS presumably corresponds to the amounts available after inhalation of LPS associated with environmental antigens, whereas the high dose corresponds to the amount present during infections with Gram-negative bacteria. It is therefore likely that the TLR4-dependent induction of T_H2 responses after inhalation of environmental LPS represents a mechanism for the pathogenesis of asthma, whereas the TLR4-dependent induction of a T_H1 response after Gram-negative infection represents a mechanism for protection from infection.

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The function of type I interferons in adaptive immunity

The type I interferon family consists of IFN-α, IFN-β, IFN-ω and IFN-λ[66]. Whereas the IFN-α gene family consists of many members, there is a single gene encoding IFN-β. Stimulation of many cell types with viruses, bacteria or TLR ligands, such as poly(I)·poly(C) LPS, CpG and imiquimod, results in the production of IFN-β and IFN-α4 (ref. 67). Factors involved in the transcription of IFN-β have been well characterized[67] and involve a complex containing the interferon regulatory factor (IRF) family member IRF-3. Once secreted, IFN-β can bind to the type I interferon receptor, a heterodimeric molecule consisting of IFNAR-1 and IFNAR-2, to activate the Janus kinase (Jak) protein tyrosine kinases Jak1 and Tyk2. This step leads to the phosphorylation of signal transducer and activator of transcription 1 (STAT1) and STAT2. STAT1–STAT2–IRF-9 forms a complex known as ISGF3, which binds to interferon-stimulated responsive element (ISRE) and turns on genes such as those encoding 2′-5′-oligoadenylate synthetase, PKR (double-stranded RNA–dependent protein kinase), IRF-7, ISG15 and IP-10. STAT1 homodimers can bind to the IFN-γ-activated site (GAS) and turn on the expression of IRF-1, which in turn binds to promoters including that for inducible nitric oxide synthetase (iNOS)[67]. Thus, the initial IFN-β and IFN-α4 secretion induced by viral infection results in a positive feedback loop via the IFN-α and IFN-β receptor (IFN-α/βR) to induce the expression of more IFN-α gene members. In contrast, LPS stimulation induces mainly IFN-β production without inducing IFN-α.

Although the function of type I interferons is most closely associated with their antiviral activities, these cytokines also have diverse effector functions in the development of adaptive immunity that are not restricted to antiviral defense. Type I interferons promote the proliferation of memory T cells and prevent T cell apoptosis[68]. Type I interferons can activate STAT4 directly and induce IFN-γ secretion from $CD8^+$ T cells in mice[69] and $CD4^+$ T cells in humans[70,71]. Further, cross-presentation of viral antigens occurs via a mechanism dependent on type I interferons. One study found that the activation of cross-priming by type I interferons was independent of $CD4^+$ T cell help but involved the direct stimulation of DCs[72]. IFN-α/β also enable B cells to undergo isotype switching and differentiation into plasma cells through the activation of DCs[73]. They are also critical activators of NK cells[74]. Finally, IFN-α/β induce DC maturation following stimulation via CpG, poly(I:C), LPS treatment or viral infection[75–77].

Given the multitude of genes that are responsive to type I interferons, it is not unexpected that they are essential in diverse aspects of adaptive immunity. It is interesting in this context that TLRs differ in their ability to induce IFN-α/β expression. TLRs 3, 4, 7 and 9 can all induce IFN-α/β production, albeit through different signaling pathways. TLRs 1, 2, 5 and 6 do not induce IFN-α/β. Because IFN-α/β functions are important in the control of adaptive immunity, it is curious that these particular TLRs do not induce their expression. Although the physiological significance of this important difference in TLR signaling is not clear, it is worth noting that any one pathogen is likely to target more that a single TLR family member. It is tempting to speculate, therefore, that for any given pathogen, a combination of TLRs that becomes activated always includes at least one TLR that can induce IFN-α/β expression.

Specialization of DC subsets in host defense

Although multiple DC subsets exist *in vivo*, the function of these cells in immune defense against various pathogens is only beginning to be unraveled. DCs are critical in the generation of multiple effector cell types, including T_H1, CTL and B cell responses. DCs are also important in the maintenance of tolerance to self antigens. How does the immune system ensure that all of the appropriate immune-inductive

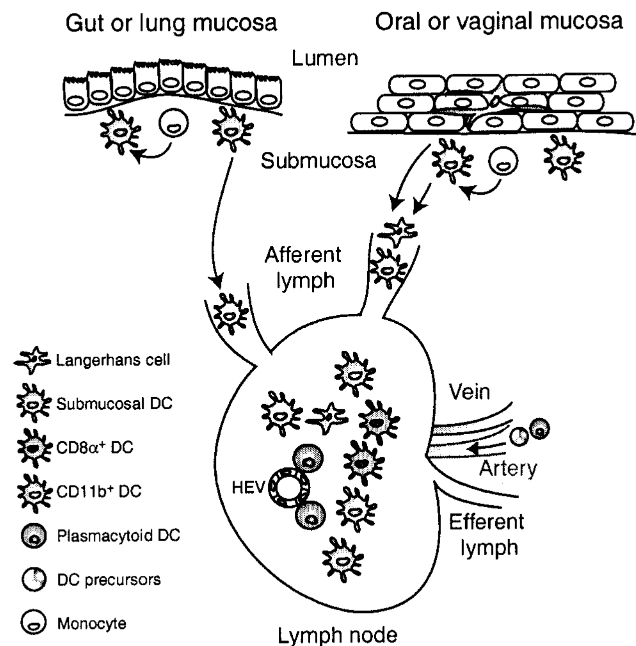

Figure 2 DC subset distribution and migration *in vivo*. In the peripheral mucosal tissues, monocytes give rise to interstitial DCs or macrophages that reside within the submucosa. In the stratified squamous epithelial layer of oral and vaginal mucosa and in the skin, Langerhans cells occupy their niche. After being stimulated, tissue DCs migrate to the lymph node to initiate naive lymphocyte activation. Within the lymph nodes, blood-derived DC subsets survey for lymph borne pathogens. The pDCs enter the lymph node via the high endothelial venules (HEVs) and 'survey' for viral pathogens. Although TLR expression of the blood-derived DCs is well characterized (**Tables 1** and **2**), TLR expression by tissue DCs is unknown. **Figure 1** shows TLR expression by the corresponding color-coordinated DC subsets.

or regulatory events are coordinated after infection? One way to accomplish this goal would be to allow one type of DC to assume flexible abilities in inducing diverse effector responses. To accomplish this, DCs must be endowed with the capacity to detect all possible pathogens by expressing the entire set of PRRs. Signals through a combination of these receptors must trigger appropriate immune responses against viruses, bacteria, parasites and fungi. But the immune systems of both humans and mice have multiple DC subsets with distinct TLR expression (**Fig. 1**), anatomical locations (**Fig. 2**) and gene expression profiles. This suggests the specialization of DC populations to deal with certain types of pathogens and to induce distinct effector cell types. Whether or not DC subsets represent distinct lineages or different maturation statuses, *ex vivo* and *in vivo* studies of these subsets have revealed a division of labor by populations of DCs in antimicrobial defense and the generation of adaptive immunity.

Within the lymph node, Peyer's patch and spleen, most DCs have an immature phenotype. These cells probably arrive in the lymphoid organs from the blood and are termed blood-derived DCs[78] (**Fig. 2**). Although it is clear that resident DCs in the secondary lymphoid tissues are capable of inducing T cell tolerance when antigen is provided in the absence of microbial stimuli[79], whether they participate in immune responses to microbial pathogens during a natural course of infection is unclear. One possibility is that at least some of the blood-derived DCs are lymphoid tissue equivalents of the peripheral tissue DCs, except that they act as sentinels of pathogens that have entered the lymph (lymph nodes) or blood (spleen). For these types of antigen presentation, different requirements exist in terms of their migratory and phagocytic activities.

The blood-derived lymphoid tissue DCs comprise many different subsets. $CD8\alpha^+$ DCs reside in the deep T cell areas of secondary lymphoid tissue. Several studies have demonstrated the critical importance of the $CD8\alpha^+$ DC subset in cross-presenting microbial antigens to $CD8^+$ T cells during viral infections with the herpes simplex[80], vaccinia and influenza viruses[81]. These cells have the potential to sense viral infection through TLR3 and TLR9 (**Fig. 1**). The $CD8\alpha^+$ DCs not only are important in cross-priming CTLs in response to viral infection but also are capable of cross-presentation of self antigens, dying cells and cell-associated foreign antigens for the induction of tolerance[79]. Thus, these cells may have a particularly enhanced ability to take up extracellular antigens, process and present them through the MHC class I pathway and serve as professional antigen-presenting cells for the generation of CTLs against pathogens that enter these lymphoid tissues or induce tolerance to self antigens in the absence of PAMPs.

The other main population of blood-derived DCs resident in lymphoid tissue are the $CD8\alpha^-CD11b^+$ DCs that are localized in the marginal zone of the spleen and in the outer edges of the paracortex in the lymph nodes. Freshly isolated $CD11b^+$ DCs from secondary lymphoid tissues induce T_H2 differentiation when transferred to a naive host[82,83]. In the Peyer's patches, $CD11b^+$ DCs reside beneath the follicle-associated epithelium, preferentially secrete IL-10 in response to bacterial stimuli and induce IL-4 and IL-10 secretion from $CD4^+$ T cells *ex vivo*[84]. These cells express mRNA for all TLRs and have the potential to respond to many types of pathogens (**Fig. 1**). Thus, in the absence of strong TLR signals, $CD11b^+$ DCs may be involved in the generation of T_H2 (refs. 82–85) or T_H3 (refs. 86,87) responses *in vivo*. However, the involvement of these cells in immune responses to microbial infection has not been formally demonstrated. One possibility is that $CD11b^+$ DCs in the Peyer's patches or mesenteric lymph nodes may be involved in the generation, activation or maintenance of IL-10–producing 'induced' T_R cells.

Unlike the lymphoid tissue DCs, several distinct populations of DCs are found in the peripheral tissues. Within the epidermal layer are the Langerhans cells that have a unique intracellular organelle known as the Birbeck granule. In response to stimulation, Langerhans cells emigrate from the epidermis to the draining lymph nodes (**Fig. 2**). Although these cells have been considered key antigen-presenting cells that present antigens in the skin, Langerhans cells do not participate in the activation of $CD4^+$ (ref. 88) or $CD8^+$ (ref. 89) T cells *in vivo* during a viral infection that targets the epithelial cells. Recent evidence indicates that Langerhans cells continually migrate from the skin to the lymph nodes in the absence of pathogen-induced maturation[90,91]. As Langerhans cells express a distinct set of PRRs[92,93], compared with the dermal or submucosal DCs that reside beneath the basement membrane, their function in the capture and presentation of bacterial, parasitic and viral pathogens may be distinct and must be examined critically.

$CD11b^+$ dermal and submucosal DCs reside beneath the basement membrane of the skin and the mucosal epithelial layer, respectively (**Fig. 2**). Once in the lymph node, the dermal and submucosal DCs can be distinguished from the resident $CD11b^+$ DCs by the moderate expression of CD205 (refs. 94,95). The $CD11b^+$ dermal DCs mediate $CD4^+$ T cell priming after antigen delivery into the skin[96]. Further, the dermal and submucosal $CD11b^+$ DCs mediate T_H1 induction after leishmania infection in the skin[97] and herpes simplex virus-2 infection in the genital mucosa[88]. Thus, the dermal and submucosal DCs represent an important tissue DC population that captures antigens, migrates to the draining lymph nodes and stimulates differentiation of T_H1 cells to various cutaneous and mucosal pathogens. Which set of TLRs are expressed and used by these cells for pathogen detection is unknown.

The mucosal surfaces lining the intestinal, respiratory and urogenital tracts represent a chief port of entry to many infectious pathogens. Consequently, the DCs that reside in the mucosal surfaces are assigned the daunting task of providing protective immunity to an onslaught of pathogens while maintaining tolerance to non-pathogenic commensal microflora and innocuous antigens. Despite their immense importance in providing antimicrobial immunity in the susceptible mucosa, the expression of TLRs, other PRRs and chemokine receptors on mucosal DCs is largely not understood.

In the intestine, DCs are found abundantly in the lamina propria. In certain circumstances, DCs extend their dendrites to the luminal surface of the intestine between the villous epithelium[98]. In the absence of infection, intestinal DCs migrate to the mesenteric lymph nodes where they prime mainly T_H3 responses[86,87]. Whether this migration is constitutive or triggered by commensal bacterial products via TLRs is not clear. However, the switch from regulatory to stimulatory DCs presumably occurs by sensing of invading pathogens via the TLRs. Because commensal bacteria can be taken up by DCs in the Peyer's patch[99], exactly how mucosal DCs avoid inducing immunity against the commensal flora also remains a mystery. Whatever the mechanism, its malfunction may trigger a series of events that can result in chronic intestinal inflammation and inflammatory bowel disease.

In the lungs, two main populations of respiratory tract DCs exist: those found in the parenchymal tissues of the lung and those in the epithelium of the conducting airways[100]. In the absence of infection, DCs isolated from the lungs express inducible costimulator ligand (ICOSL) and secrete IL-10, selectively inducing regulatory T cells[101]. Induction of optimal T_H1 responses by lung DCs requires exposure to microbial pathogens such as influenza virus infection[102,103]. Although the lung DCs support asthmatic diseases at both the

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inductive and maintenance phases[100], the requirement for PRR signals in the allergic inflammatory lesions is not yet fully understood. As mentioned previously, in the presence of low levels of LPS, TLR4 is necessary for the induction of T_H2 responses to inhaled antigens in a mouse model of allergic sensitization through the activation of DCs[65].

Distinct DC precursors and subsets continuously circulate in peripheral blood (**Fig. 2**). The monocytes comprise a large fraction of peripheral blood mononuclear cells. Once in tissue, monocytes can differentiate into either myeloid DCs or tissue macrophages. The differentiation of monocytes is regulated by their interaction with extracellular matrices associated with the endothelial cells[104]. Because human blood monocytes express TLRs 1, 2, 4, 5, 6 and 8 (**Fig. 1**), they are probably specialized in the recognition of a variety of pathogens in the blood and in peripheral tissues.

In peripheral blood, pDCs constitute less than 1% of the mononuclear cells. During viral infection, pDCs are recruited to the inflamed lymph node via the high endothelial venules[105] and provide a source of type I interferons. A critical *in vivo* function for these cells in the secretion of IFN-α was first demonstrated by antibody depletion of pDCs with anti–Ly6G/C. This treatment resulted in a dramatic decrease in serum IFN-α concentrations after systemic infection with the virus MCMV[106]. The unique capacity of pDCs to secrete high concentrations of type I interferons during viral infection is related at least in part to their ability to recognize viruses through TLRs. pDCs express both TLR7 and TLR9 and recognize single-stranded RNA[4–6] and double-stranded DNA[10,43] viruses, respectively (**Fig. 1**). Although pDCs undergo rapid apoptosis *in vitro*, their survival is enhanced in response to IL-3 (ref. 107) and Flt-3L[108,109] and they differentiate in response to viruses, CpG[33,34] or IL-3 and CD40L[110]. Although pDCs were originally shown to induce T_H2 cytokine secretion by naive $CD4^+$ T cells[107], in the presence of viruses or CD40 stimulation, pDCs can induce T_H1 differentiation[40,111]. Furthermore, these cells can also induce T_R cells[112,113]. Despite this *in vitro* evidence for T cell activation, *in vivo* studies show the lack of direct participation of pDCs in antigen presentation to naive $CD4^+$ or $CD8^+$ T lymphocytes[78,89,114]. Thus, whether pDCs are directly or indirectly involved in T cell differentiation or regulation *in vivo* remains to be determined. A recent study showed that pDCs also promote differentiation of B cells into plasma cells[115]. Therefore, pDCs have an important function in antiviral defense via the secretion of large amount of type I interferons in local lymphoid tissues and the prevention of viral replication, and they contribute to many other pathways induced by the type I interferon system, as described above.

TLRs in B cell activation and antibody production

B lymphocytes have a somewhat unusual status in the immune system because they express both clonally rearranged antigen receptors and nonclonal PPRs, most notably TLRs. Stimulation of TLRs expressed on B cells can lead to polyclonal activation and production of low-affinity immunoglobulin M (IgM) antibodies. B1 cells of the peritoneal cavity and marginal zone B cells are particularly sensitive to TLR ligands and participate in T cell–independent immediate antibody responses that are typically directed against common bacterial components, such as phosphatidylcholine[116]. Because the polyclonal activation of B2 cells may potentially result in the production of autoreactive antibodies, several mechanisms have evolved to regulate B cell activation, depending on the specificity of their immunoglobulin receptor. Thus B cells that express autoreactive membrane immunoglobulin induce a tolerogenic signaling pathway that inhibits TLR-induced B cell activation[117]. In contrast, the physiological importance of polyclonal activation by TLR ligands has been demonstrated for memory B cells. Human memory B cells produced antibodies in response to CpG stimulation independently of antigen-specific cognate T cell help[118]. Because memory B cells have already been selected for their specificity, their polyclonal activation is not expected to result in autoimmunity. Therefore, it seems that naive and memory B cells may respond to TLR stimulation differently to ensure both self-tolerance and a rapid response to reinfection.

Concluding remarks

TLRs sense microbial infection and engage multiple mechanisms that control the initiation of adaptive immune responses. DCs are the key cell type that couples TLR-mediated innate immune recognition to the initiation of T cell and B cell activation. Recent studies suggest that DCs may be specialized to accomplish distinct functions in the course of the immune response, such that multiple DC subtypes are engaged in response to a single infection. This functional specialization may explain the differential TLR expression patterns in different DC subsets. Type I interferons, which are best known for their antiviral activities, have many essential functions in the control of adaptive immunity. Future work will need to address the biological importance of the known differences in TLR function, including expression patterns, ligand specificities and the differentially engaged signaling pathways. How all these characteristics of individual TLRs translate into the induction of protective immunity to a given pathogen still remains a mystery.

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COMPETING INTERESTS STATEMENT
The authors declare that they have no competing financial interests.

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1. Janeway, C.A. Jr. & Medzhitov, R. Introduction: the role of innate immunity in the adaptive immune response. *Semin. Immunol.* **10**, 349–350 (1998).
2. Dangl, J.L. & Jones, J.D. Plant pathogens and integrated defence responses to infection. *Nature* **411**, 826–833 (2001).
3. Takeda, K., Kaisho, T. & Akira, S. Toll-like receptors. *Annu. Rev. Immunol.* **21**, 335–376 (2003).
4. Heil, F. *et al.* Species-specific recognition of single-stranded RNA via toll-like receptor 7 and 8. *Science* **303**, 1526–1529 (2004).
5. Diebold, S.S., Kaisho, T., Hemmi, H., Akira, S. & Reis, E.S.C. Innate antiviral responses by means of TLR7-mediated recognition of single-stranded RNA. *Science* **303**, 1529–1531 (2004).
6. Lund, J.M. *et al.* Recognition of single-stranded RNA viruses by Toll-like receptor 7. *Proc. Natl. Acad. Sci. USA* **101**, 5598–5603 (2004).
7. Heil, F. *et al.* The Toll-like receptor 7 (TLR7)-specific stimulus loxoribine uncovers a strong relationship within the TLR7, 8 and 9 subfamily. *Eur. J. Immunol.* **33**, 2987–2997 (2003).
8. Lee, J. *et al.* Molecular basis for the immunostimulatory activity of guanine nucleoside analogs: activation of Toll-like receptor 7. *Proc. Natl. Acad. Sci. USA* **100**, 6646–6651 (2003).
9. Matsumoto, M. *et al.* Subcellular localization of toll-like receptor 3 in human dendritic cells. *J. Immunol.* **171**, 3154–3162 (2003).
10. Lund, J., Sato, A., Akira, S., Medzhitov, R. & Iwasaki, A. Toll-like receptor 9-mediated recognition of Herpes simplex virus-2 by plasmacytoid dendritic cells. *J. Exp. Med.* **198**, 513–520 (2003).
11. Leadbetter, E.A. *et al.* Chromatin-IgG complexes activate B cells by dual engagement of IgM and Toll-like receptors. *Nature* **416**, 603–607 (2002).
12. Laudanna, C., Kim, J.Y., Constantin, G. & Butcher, E. Rapid leukocyte integrin activation by chemokines. *Immunol. Rev.* **186**, 37–46 (2002).
13. Huang, Q. *et al.* The plasticity of dendritic cell responses to pathogens and their components. *Science* **294**, 870–875 (2001).
14. Mantovani, A. The chemokine system: redundancy for robust outputs. *Immunol. Today* **20**, 254–257 (1999).
15. Hayashi, F., Means, T.K. & Luster, A.D. Toll-like receptors stimulate human neutrophil function. *Blood* **102**, 2660–2669 (2003).
16. Neufert, C. *et al.* Mycobacterium tuberculosis 19-kDa lipoprotein promotes neutrophil activation. *J. Immunol.* **167**, 1542–1549 (2001).

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17. Hornung, V. *et al.* Quantitative expression of toll-like receptor 1-10 mRNA in cellular subsets of human peripheral blood mononuclear cells and sensitivity to CpG oligodeoxynucleotides. *J. Immunol.* **168**, 4531–4537 (2002).
18. Nagase, H. *et al.* Expression and function of Toll-like receptors in eosinophils: activation by Toll-like receptor 7 ligand. *J. Immunol.* **171**, 3977–3982 (2003).
19. McCurdy, J.D., Olynych, T.J., Maher, L.H. & Marshall, J.S. Cutting edge: distinct Toll-like receptor 2 activators selectively induce different classes of mediator production from human mast cells. *J. Immunol.* **170**, 1625–1629 (2003).
20. Supajatura, V. *et al.* Differential responses of mast cell Toll-like receptors 2 and 4 in allergy and innate immunity. *J. Clin. Invest.* **109**, 1351–1359 (2002).
21. Supajatura, V. *et al.* Protective roles of mast cells against enterobacterial infection are mediated by Toll-like receptor 4. *J. Immunol.* **167**, 2250–2256 (2001).
22. Fan, J. & Malik, A.B. Toll-like receptor-4 (TLR4) signaling augments chemokine-induced neutrophil migration by modulating cell surface expression of chemokine receptors. *Nat. Med.* **9**, 315–321 (2003).
23. Ashkar, A.A., Bauer, S., Mitchell, W.J., Vieira, J. & Rosenthal, K.L. Local delivery of CpG oligodeoxynucleotides induces rapid changes in the genital mucosa and inhibits replication, but not entry, of herpes simplex virus type 2. *J. Virol.* **77**, 8948–8956 (2003).
24. Zhang, D. *et al.* A toll-like receptor that prevents infection by uropathogenic bacteria. *Science* **303**, 1522–1526 (2004).
25. Kagnoff, M.F. & Eckmann, L. Epithelial cells as sensors for microbial infection. *J. Clin. Invest.* **100**, 6–10 (1997).
26. Hertz, C.J. *et al.* Activation of Toll-like receptor 2 on human tracheobronchial epithelial cells induces the antimicrobial peptide human beta defensin-2. *J. Immunol.* **171**, 6820–6826 (2003).
27. Andonegui, G. *et al.* Endothelium-derived Toll-like receptor-4 is the key molecule in LPS-induced neutrophil sequestration into lungs. *J. Clin. Invest.* **111**, 1011–1020 (2003).
28. Schilling, J.D., Martin, S.M., Hung, C.S., Lorenz, R.G. & Hultgren, S.J. Toll-like receptor 4 on stromal and hematopoietic cells mediates innate resistance to uropathogenic Escherichia coli. *Proc. Natl. Acad. Sci. USA* **100**, 4203–4208 (2003).
29. Banchereau, J. & Steinman, R.M. Dendritic cells and the control of immunity. *Nature* **392**, 245–252 (1998).
30. Schnare, M. *et al.* Toll-like receptors control activation of adaptive immune responses. *Nat. Immunol.* **2**, 947–950 (2001).
31. Palliser, D., Ploegh, H. & Boes, M. Myeloid differentiation factor 88 is required for cross-priming *in vivo*. *J. Immunol.* **172**, 3415–3421 (2004).
32. Jarrossay, D., Napolitani, G., Colonna, M., Sallusto, F. & Lanzavecchia, A. Specialization and complementarity in microbial molecule recognition by human myeloid and plasmacytoid dendritic cells. *Eur. J. Immunol.* **31**, 3388–3393 (2001).
33. Kadowaki, N. *et al.* Subsets of human dendritic cell precursors express different toll-like receptors and respond to different microbial antigens. *J. Exp. Med.* **194**, 863–869 (2001).
34. Krug, A. *et al.* Toll-like receptor expression reveals CpG DNA as a unique microbial stimulus for plasmacytoid dendritic cells which synergizes with CD40 ligand to induce high amounts of IL-12. *Eur. J. Immunol.* **31**, 3026–3037 (2001).
35. Ito, T. *et al.* Interferon-α and interleukin-12 are induced differentially by Toll-like receptor 7 ligands in human blood dendritic cell subsets. *J. Exp. Med.* **195**, 1507–1512 (2002).
36. Visintin, A. *et al.* Regulation of Toll-like receptors in human monocytes and dendritic cells. *J. Immunol.* **166**, 249–255 (2001).
37. Muzio, M. *et al.* Differential expression and regulation of toll-like receptors (TLR) in human leukocytes: selective expression of TLR3 in dendritic cells. *J. Immunol.* **164**, 5998–6004 (2000).
38. Edwards, A.D. *et al.* Toll-like receptor expression in murine DC subsets: lack of TLR7 expression by CD8 α^+ DC correlates with unresponsiveness to imidazoquinolines. *Eur. J. Immunol.* **33**, 827–833 (2003).
39. Doxsee, C.L. *et al.* The immune response modifier and Toll-like receptor 7 agonist S-27609 selectively induces IL-12 and TNF-α production in $CD11c^+CD11b^+CD8^-$ dendritic cells. *J. Immunol.* **171**, 1156–1163 (2003).
40. Boonstra, A. *et al.* Flexibility of mouse classical and plasmacytoid-derived dendritic cells in directing T helper type 1 and 2 cell development: dependency on antigen dose and differential toll-like receptor ligation. *J. Exp. Med.* **197**, 101–109 (2003).
41. Underhill, D.M. *et al.* The Toll-like receptor 2 is recruited to macrophage phagosomes and discriminates between pathogens. *Nature* **401**, 811–815 (1999).
42. Ahmad-Nejad, P. *et al.* Bacterial CpG-DNA and lipopolysaccharides activate Toll-like receptors at distinct cellular compartments. *Eur. J. Immunol.* **32**, 1958–1968 (2002).
43. Krug, A. *et al.* Herpes simplex virus type 1 (HSV-1) activates murine natural interferon-producing cells (IPC) through Toll-like receptor 9. *Blood* **103**, 1433–1437 (2003).
44. Sallusto, F. *et al.* Rapid and coordinated switch in chemokine receptor expression during dendritic cell maturation. *Eur. J. Immunol.* **28**, 2760–2769 (1998).
45. Dieu, M.C. *et al.* Selective recruitment of immature and mature dendritic cells by distinct chemokines expressed in different anatomic sites. *J. Exp. Med.* **188**, 373–386 (1998).
46. Forster, R. *et al.* CCR7 coordinates the primary immune response by establishing functional microenvironments in secondary lymphoid organs. *Cell* **99**, 23–33 (1999).
47. Gunn, M.D. *et al.* Mice lacking expression of secondary lymphoid organ chemokine have defects in lymphocyte homing and dendritic cell localization. *J. Exp. Med.* **189**, 451–460 (1999).
48. Constant, S.L. & Bottomly, K. Induction of Th1 and Th2 $CD4^+$ T cell responses: the alternative approaches. *Annu. Rev. Immunol.* **15**, 297–322 (1997).
49. Janssen, E.M. *et al.* $CD4^+$ T cells are required for secondary expansion and memory in $CD8^+$ T lymphocytes. *Nature* **421**, 852–856 (2003).
50. Shedlock, D.J. & Shen, H. Requirement for CD4 T cell help in generating functional CD8 T cell memory. *Science* **300**, 337–339 (2003).
51. Sun, J.C. & Bevan, M.J. Defective CD8 T cell memory following acute infection without CD4 T cell help. *Science* **300**, 339–342 (2003).
52. Bourgeois, C., Veiga-Fernandes, H., Joret, A.M., Rocha, B. & Tanchot, C. CD8 lethargy in the absence of CD4 help. *Eur. J. Immunol.* **32**, 2199–2207 (2002).
53. Medzhitov, R. Toll-like receptors and innate immunity. *Nat. Rev. Immunol.* **1**, 135–145 (2001).
54. Hemmi, H., Kaisho, T., Takeda, K. & Akira, S. The roles of Toll-like receptor 9, MyD88, and DNA-dependent protein kinase catalytic subunit in the effects of two distinct CpG DNAs on dendritic cell subsets. *J. Immunol.* **170**, 3059–3064 (2003).
55. Hori, S., Nomura, T. & Sakaguchi, S. Control of regulatory T cell development by the transcription factor Foxp3. *Science* **299**, 1057–1061 (2003).
56. Fontenot, J.D., Gavin, M.A. & Rudensky, A.Y. Foxp3 programs the development and function of $CD4^+CD25^+$ regulatory T cells. *Nat. Immunol.* **4**, 330–336 (2003).
57. Khattri, R., Cox, T., Yasayko, S.A. & Ramsdell, F. An essential role for Scurfin in $CD4^+CD25^+$ T regulatory cells. *Nat. Immunol.* **4**, 337–342 (2003).
58. Pasare, C. & Medzhitov, R. Toll pathway-dependent blockade of $CD4^+CD25^+$ T cell-mediated suppression by dendritic cells. *Science* **299**, 1033–1036 (2003).
59. Yang, Y., Huang, C.T., Huang, X. & Pardoll, D.M. Persistent Toll-like receptor signals are required for reversal of regulatory T cell-mediated CD8 tolerance. *Nat. Immunol.* **5**, 508–515 (2004).
60. Trinchieri, G. Interleukin-12 and the regulation of innate resistance and adaptive immunity. *Nat. Rev. Immunol.* **3**, 133–146 (2003).
61. Kaisho, T. *et al.* Endotoxin can induce MyD88-deficient dendritic cells to support T(h)2 cell differentiation. *Int. Immunol.* **14**, 695–700 (2002).
62. Redecke, V. *et al.* Cutting edge: Activation of toll-like receptor 2 induces a Th2 immune response and promotes experimental asthma. *J. Immunol.* **172**, 2739–2743 (2004).
63. Pulendran, B. *et al.* Lipopolysaccharides from distinct pathogens induce different classes of immune responses *in vivo*. *J. Immunol.* **167**, 5067–5076 (2001).
64. Agrawal, S. *et al.* Cutting edge: different Toll-like receptor agonists instruct dendritic cells to induce distinct Th responses via differential modulation of extracellular signal-regulated kinase-mitogen-activated protein kinase and c-Fos. *J. Immunol.* **171**, 4984–4989 (2003).
65. Eisenbarth, S.C. *et al.* Lipopolysaccharide-enhanced, toll-like receptor 4-dependent T helper cell type 2 responses to inhaled antigen. *J. Exp. Med.* **196**, 1645–1651 (2002).
66. Katze, M.G., He, Y. & Gale, M. Jr. Viruses and interferon: a fight for supremacy. *Nat. Rev. Immunol.* **2**, 675–687 (2002).
67. Taniguchi, T. & Takaoka, A. The interferon-α/β system in antiviral responses: a multimodal machinery of gene regulation by the IRF family of transcription factors. *Curr. Opin. Immunol.* **14**, 111–116 (2002).
68. Tough, D.F., Sun, S., Zhang, X. & Sprent, J. Stimulation of naive and memory T cells by cytokines. *Immunol. Rev.* **170**, 39–47 (1999).
69. Nguyen, K.B. *et al.* Critical role for STAT4 activation by type 1 interferons in the interferon-γ response to viral infection. *Science* **297**, 2063–2066 (2002).
70. Sareneva, T., Matikainen, S., Kurimoto, M. & Julkunen, I. Influenza A virus-induced IFN-α/β and IL-18 synergistically enhance IFN-γ gene expression in human T cells. *J. Immunol.* **160**, 6032–6038 (1998).
71. Rogge, L. *et al.* The role of Stat4 in species-specific regulation of Th cell development by type I IFNs. *J. Immunol.* **161**, 6567–6574 (1998).
72. Le Bon, A. *et al.* Cross-priming of $CD8^+$ T cells stimulated by virus-induced type I interferon. *Nat. Immunol.* **4**, 1009–1015 (2003).
73. Le Bon, A. *et al.* Type i interferons potently enhance humoral immunity and can promote isotype switching by stimulating dendritic cells *in vivo*. *Immunity* **14**, 461–470 (2001).
74. Biron, C.A., Nguyen, K.B., Pien, G.C., Cousens, L.P. & Salazar-Mather, T.P. Natural killer cells in antiviral defense: function and regulation by innate cytokines. *Annu. Rev. Immunol.* **17**, 189–220 (1999).
75. Hoshino, K., Kaisho, T., Iwabe, T., Takeuchi, O. & Akira, S. Differential involvement of IFN-β in Toll-like receptor-stimulated dendritic cell activation. *Int. Immunol.* **14**, 1225–1231 (2002).
76. Honda, K. *et al.* Selective contribution of IFN-α/β signaling to the maturation of dendritic cells induced by double-stranded RNA or viral infection. *Proc. Natl. Acad. Sci. USA* **100**, 10872–10877 (2003).
77. Hoebe, K. *et al.* Upregulation of costimulatory molecules induced by lipopolysaccharide and double-stranded RNA occurs by Trif-dependent and Trif-independent pathways. *Nat. Immunol.* **4**, 1223–1229 (2003).
78. Itano, A.A. *et al.* Distinct dendritic cell populations sequentially present antigen to CD4 T cells and stimulate different aspects of cell-mediated immunity. *Immunity* **19**, 47–57 (2003).
79. Steinman, R.M., Hawiger, D. & Nussenzweig, M.C. Tolerogenic dendritic cells. *Annu. Rev. Immunol.* **21**, 685–711 (2003).
80. Smith, C.M. *et al.* Cutting Edge: Conventional CD8α^+ Dendritic Cells Are Preferentially Involved in CTL Priming After Footpad Infection with Herpes Simplex

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Virus-1. *J. Immunol.* **170**, 4437–4440 (2003).
81. Belz, G.T. *et al.* Cutting edge: conventional CD8α^+ dendritic cells are generally involved in priming CTL immunity to viruses. *J. Immunol.* **172**, 1996–2000 (2004).
82. Pulendran, B. *et al.* Distinct dendritic cell subsets differentially regulate the class of immune response *in vivo. Proc. Natl. Acad. Sci. USA* **96**, 1036–1041 (1999).
83. Maldonado, L.R. *et al.* CD8α^+ and CD8α- Subclasses of Dendritic Cells Direct the Development of Distinct T Helper Cells *In vivo. J. Exp. Med.* **189**, 587–592 (1999).
84. Iwasaki, A. & Kelsall, B.L. Unique functions of CD11b$^+$, CD8 α^+, and double-negative Peyer's patch dendritic cells. *J. Immunol.* **166**, 4884–4890 (2001).
85. Moser, M. & Murphy, K.M. Dendritic cell regulation of T_H1-T_H2 development. *Nat. Immunol.* **1**, 199–205 (2000).
86. Akbari, O., DeKruyff, R.H. & Umetsu, D.T. Pulmonary dendritic cells producing IL-10 mediate tolerance induced by respiratory exposure to antigen. *Nat. Immunol.* **2**, 725–731 (2001).
87. Alpan, O. Oral tolerance and gut-oriented immune response to dietary proteins. *Curr. Allergy Asthma Rep.* **1**, 572–577 (2001).
88. Zhao, X. *et al.* Vaginal submucosal dendritic cells, but not Langerhans cells, induce protective Th1 responses to herpes simplex virus-2. *J. Exp. Med.* **197**, 153–162 (2003).
89. Allan, R.S. *et al.* Epidermal viral immunity induced by CD8α^+ dendritic cells but not by Langerhans cells. *Science* **301**, 1925–1928 (2003).
90. Randolph, G.J. Is maturation required for Langerhans cell migration? *J. Exp. Med.* **196**, 413–416 (2002).
91. Geissmann, F. *et al.* Accumulation of immature Langerhans cells in human lymph nodes draining chronically inflamed skin. *J. Exp. Med.* **196**, 417–430 (2002).
92. Valladeau, J. *et al.* Langerin, a novel C-type lectin specific to Langerhans cells, is an endocytic receptor that induces the formation of Birbeck granules. *Immunity* **12**, 71–81 (2000).
93. Valladeau, J. *et al.* Identification of mouse langerin/CD207 in Langerhans cells and some dendritic cells of lymphoid tissues. *J. Immunol.* **168**, 782–792 (2002).
94. Henri, S. *et al.* The dendritic cell populations of mouse lymph nodes. *J. Immunol.* **167**, 741–748 (2001).
95. Anjuere, F. *et al.* Definition of dendritic cell subpopulations present in the spleen, Peyer's patches, lymph nodes, and skin of the mouse. *Blood* **93**, 590–598 (1999).
96. Itano, A.A. *et al.* Distinct dendritic cell populations sequentially present a subcutaneous antigen to CD4 T cells and stimulate different aspects of cell-mediated immunity. *Immunity* (in the press).
97. Filippi, C. *et al.* CD4$^+$ T cell polarization in mice is modulated by strain-specific MHC-independent differences within dendritic cells. *J. Exp. Med.* **198**, 201–209 (2003).
98. Rescigno, M. *et al.* Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. *Nat. Immunol.* **2**, 361–367 (2001).
99. Macpherson, A.J. & Uhr, T. Induction of protective IgA by intestinal dendritic cells carrying commensal bacteria. *Science* **303**, 1662–1665 (2004).
100. Lambrecht, B.N. & Hammad, H. Taking our breath away: dendritic cells in the pathogenesis of asthma. *Nat. Rev. Immunol.* **3**, 994–1003 (2003).
101. Akbari, O., Stock, P., DeKruyff, R.H. & Umetsu, D.T. Mucosal tolerance and immunity: regulating the development of allergic disease and asthma. *Int. Arch. Allergy Immunol.* **130**, 108–118 (2003).
102. Legge, K.L. & Braciale, T.J. Accelerated migration of respiratory dendritic cells to the regional lymph nodes is limited to the early phase of pulmonary infection. *Immunity* **18**, 265–277 (2003).
103. Dahl, M.E., Dabbagh, K., Liggitt, D., Kim, S. & Lewis, D.B. Viral-induced T helper type 1 responses enhance allergic disease by effects on lung dendritic cells. *Nat. Immunol.* **5**, 337–343 (2004).
104. Randolph, G.J., Beaulieu, S., Lebecque, S., Steinman, R.M. & Muller, W.A. Differentiation of monocytes into dendritic cells in a model of transendothelial trafficking. *Science* **282**, 480–483 (1998).
105. Cella, M. *et al.* Plasmacytoid monocytes migrate to inflamed lymph nodes and produce large amounts of type I interferon. *Nat. Med.* **5**, 919–923 (1999).
106. Asselin-Paturel, C. *et al.* Mouse type I IFN-producing cells are immature APCs with plasmacytoid morphology. *Nat. Immunol.* **2**, 1144–1150 (2001).
107. Rissoan, M.C. *et al.* Reciprocal control of T helper cell and dendritic cell differentiation. *Science* **283**, 1183–1186 (1999).
108. Gilliet, M. *et al.* The development of murine plasmacytoid dendritic cell precursors is differentially regulated by FLT3-ligand and granulocyte/macrophage colony-stimulating factor. *J. Exp. Med.* **195**, 953–958 (2002).
109. Brawand, P. *et al.* Murine plasmacytoid pre-dendritic cells generated from flt3 ligand-supplemented bone marrow cultures are immature APCs. *J. Immunol.* **169**, 6711–6719 (2002).
110. Grouard, G. *et al.* The enigmatic plasmacytoid T cells develop into dendritic cells with interleukin (IL)-3 and CD40-ligand. *J. Exp. Med.* **185**, 1101–1111 (1997).
111. Cella, M., Facchetti, F., Lanzavecchia, A. & Colonna, M. Plasmacytoid dendritic cells activated by influenza virus and CD40L drive a potent Th1 polarization. *Nat. Immunol.* **4**, 305–310 (2000).
112. Martin, P. *et al.* Characterization of a new subpopulation of mouse CD8α^+ B220$^+$ dendritic cells endowed with type 1 interferon production capacity and tolerogenic potential. *Blood* **100**, 383–390 (2002).
113. Gilliet, M. & Liu, Y.J. Human plasmacytoid-derived dendritic cells and the induction of T-regulatory cells. *Hum. Immunol.* **63**, 1149–1155 (2002).
114. Filippi, C. *et al.* CD4$^+$ T cell polarization in mice is modulated by strain-specific major histocompatibility complex-independent differences within dendritic cells. *J. Exp. Med.* **198**, 201–209 (2003).
115. Jego, G. *et al.* Plasmacytoid dendritic cells induce plasma cell differentiation through type I interferon and interleukin 6. *Immunity* **19**, 225–234 (2003).
116. Martin, F. & Kearney, J.F. Marginal-zone B cells. *Nat. Rev. Immunol.* **2**, 323–335 (2002).
117. Rui, L., Vinuesa, C.G., Blasioli, J. & Goodnow, C.C. Resistance to CpG DNA-induced autoimmunity through tolerogenic B cell antigen receptor ERK signaling. *Nat. Immunol.* **4**, 594–600 (2003).
118. Bernasconi, N.L., Traggiai, E. & Lanzavecchia, A. Maintenance of serological memory by polyclonal activation of human memory B cells. *Science* **298**, 2199–2202 (2002).

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