How dendritic cells and microbes interact to elicit or subvert protective immune responses

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B and T lymphocytes recognize antigens with high specificity, but neither initiate immune responses, nor decide their types. These functions rest upon dendritic cells (DCs), which can determine and maintain Th1/Th2 polarization. Immune responses are thus dependent on the DC subset, the receptors that recognize each pathogen and the microenvironment. Microbes employ an array of mechanisms to evade and disrupt DC functions; some even hijack DCs for transport around the body. Our progress in the understanding of DC physiology will hopefully help us create the necessary vaccines to counteract the infectious agents that still plague mankind.

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Abbreviations

CMV	cytomegalovirus
DC	dendritic cell
EBV	Epstein–Barr virus
HCMV	human CMV
HPC	hematopoietic progenitor cell
HSV-1	herpes simplex virus 1
intDC	interstitial DC
LC	Langerhans cell
LCMV	lymphocytic choriomeningitis virus
LPS	lipopolysaccharide
mDC	myeloid DC
MV	measles virus
pDC	plasmacytoid DC
TLR	Toll-like receptor
Tr cell	T regulatory cell
VSV	vesicular stomatitis virus

Introduction

It took hundreds of millions of years of evolution to endow the upper vertebrates with a system that efficiently copes with a myriad of microbes, including viruses, bacteria, fungi and parasites. The host–microbe relationship is a dynamic process in which the microbe attempts to minimize its visibility, to ensure survival, whereas the host attempts to prevent and eradicate infection with a minimal damage to self. Antimicrobial protection is ensured by the coordinated action of the innate and adaptive immune systems [1•].

The innate immune system is composed of two elements: firstly, cells with complementary antimicrobial functions, including epithelial cells, neutrophils, NK and NKT cells, macrophages and dendritic cells (DCs); and secondly, proteins such as cytokines that are produced by the cells of immune system or proteins such as complement factors that are produced by nonimmune cells.

The first line of defense is epithelial surfaces of the airways and gastrointestinal tract, which face a mixture of antigens, mostly ubiquitous nonpathogenic antigens from plants (pollen), food and commensal microbes, interspersed with pathogenic microbes (viruses and bacteria). In the conductive airways, the majority of the antigens are removed by the overlying mucociliary escalator. Furthermore, respiratory epithelia are coated with a thin layer of secretions, which contain antimicrobial agents, for example lysozyme, defensins and bactericidal/permeability-increasing protein [2•]. Another protection level is provided by the tight junctions that connect the epithelial cells, forming a formidable barrier that prevents the entry of microbes and their products. A small proportion of incoming microbes that enter at sites of microlesions is then handled by antigen-presenting cells (APCs), which are mostly DCs [3].

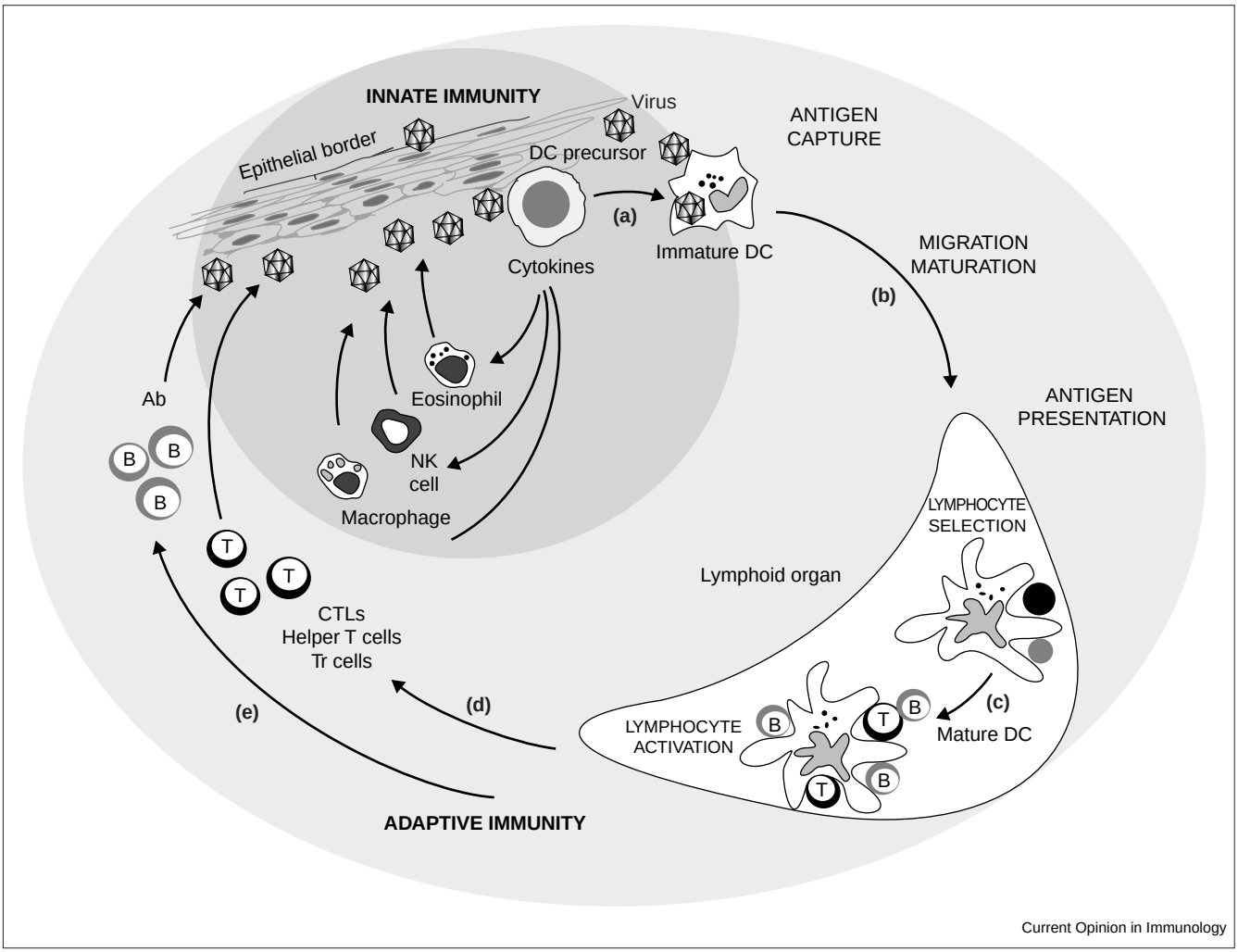
Thus, when a pathogen invades a tissue, the immune system faces several challenges. First, it must sense a pathogen and then deliver an appropriate immune response. Indeed, the type of immune response mounted by the adaptive immune system, composed of B and T lymphocytes, can actually be a matter of life or death. For example, the tuberculoid form of leprosy is characterized by a 'type 1' response and low morbidity, but its lepromatous form, which is characterized by a 'type 2' response, often kills the host.

Basics of dendritic cell biology

Although B and T lymphocytes recognize antigens with high specificity, they do not initiate an immune response, nor do they decide its type. These functions rest upon DCs (reviewed in [3,4,5•]). DCs sit in an immature state, like watchdogs, in all peripheral tissues. These immature DCs behave like 'immunological sensors' in perceiving microbial signals, integrating and processing them and then conveying the message to lymphocytes (Figure 1). There is now substantial evidence that immature DCs have also a role in the maintenance of peripheral tolerance to self antigens [6•].

Once they have sensed a microbe, DCs undergo considerable changes, collectively called maturation, that occur while they migrate from the peripheral tissues into the draining lymph nodes. Meanwhile, they process the microbial products to present their peptides — as complexes with MHC proteins together with costimulatory molecules — to lymphocytes. Whereas B cells can directly recognize native

Figure 1



The life cycle of DCs. (a) Circulating precursor DCs enter tissues as immature DCs. They can directly encounter pathogens (e.g. a virus is shown here), which induce secretion of cytokines such as IFN-α, which in turn activate effector cells of innate immunity such as eosinophils, macrophages and NK cells. (b) Following antigen capture, immature DCs migrate to lymphoid organs where, after maturation, they display peptide–MHC complexes, which allow antigen presentation and selection of rare circulating antigen-specific lymphocytes. (c) These activated T cells help DCs in terminal maturation, which allows lymphocyte expansion and differentiation to mediate adaptive immunity. (d) Activated T lymphocytes (cytotoxic T lymphocytes [CTLs], helper T cells and Tr cells) traverse inflamed epithelia and reach the injured tissue, where they eliminate microbes and/or microbe-infected cells. (e) Activated B cells migrate into various areas where they mature into plasma cells that produce antibody (Ab) that neutralizes the initial pathogen.

antigens, $CD8^+$ and $CD4^+$ T cells recognize antigen fragments bound to MHC class I and II molecules expressed on DCs, respectively. The CD1 molecules can also present microbial nonprotein antigens to T/NK/NKT cells [7]. DCs are also able to influence B cell proliferation, differentiation and isotype switching [8•].

Thus, different microbial components can be presented to different immune effectors thereby providing a broad immune response. Such diversification of immune response may explain how vertebrates can survive the thousands of threatening microbes. Recent studies have emphasized the critical role of DCs in the mobilization of innate immunity, particularly of NK cells [9•]. Such mobilization occurs via multiple pathways: these include, firstly, chemokine-mediated attraction; secondly, cytokine (IL-12 and IFN-α)-mediated activation; and thirdly, contact-dependent activation [10•–12•].

We will review herein how DCs handle microbes to elicit a protective immune response and how pathogens, in their quest for survival, have evolved to either avoid or — in a Mephistophelean way — subvert DCs.

Dendritic cell subsets

Human subsets

Skin epidermis contains Langerhans cells (LCs) whereas dermis contains interstitial (dermal) DCs (intDCs). These

two subsets emerge in cultures of CD34+ hematopoietic progenitor cells (HPCs) driven by GM-CSF and TNF-α [13], and display common as well as unique functions. For example, intDCs, but not LCs, express high levels of nonspecific esterases and can induce the differentiation of naïve B cells into plasma cells.

Two subsets of DC precursors circulate in the blood: firstly, lineage-negative CD11c+ myeloid DCs (mDCs), which derive from monocytes; and secondly, CD11c–IL-3Rα+ plasmacytoid DCs (pDCs), which are possibly related to the lymphoid lineage [4]. *In vitro*, pDCs are dependent on cytokines for survival (IL-3 and IFN-α) and maturation (TNF-α and CD40L). They express lymphoid antigens [14] and produce large amounts of type I interferon in response to many viruses [15,16]. pDCs are also found in lymphoid organs (thymus, bone marrow, spleen, tonsils and lymph nodes), and are increased in inflamed tissues such as lupus skin [17] or allergen-challenged nasal mucosa [18].

As we will discuss later, different DC subsets express different receptors for microbial products. This permits them to initiate responses to a large variety of microbes and to generate different immune responses to a single microbe, thus increasing the chances of successfully controlling the microbial invasion.

Murine subsets

Early studies classified mouse DCs into two major subsets — the 'myeloid' CD8– subset and the 'lymphoid' CD8+ subset — which differ in phenotype, localization and function. CD8α+ DCs are localized in the T-cell-rich areas of the spleen and lymph nodes whereas CD8α– DCs are in the marginal zones.

Recent studies challenged this dual-subset view, a detailed account being provided in a recent review [19•]. In particular, CD8 represents an activation marker of LCs, and both committed hematopoietic lymphoid and myeloid progenitors yield both CD8+ and CD8– DCs [19•].

Most recently, a common precursor population, yielding CD8+ and CD8– murine DCs but devoid of myeloid or lymphoid differentiation potential, has been characterized [20]. The mouse equivalent to human pDCs has been identified based on its ability to produce type I interferon [21••–23••] in response to many viruses including murine cytomegalovirus (CMV) [24•] and vesicular stomatitis virus (VSV) [25•]. Yet, not every virus acts through pDCs. For example, lymphocytic choriomeningitis virus (LCMV) induces the release of interferon independently of pDCs or NK cells, possibly from epithelial cells and fibroblasts [24•].

Dendritic cells tune the type of immune response

Type 1 T cells (Th1 cells), which secrete IFN-γ, and type 2 T cells (Th2 cells), which secrete IL-4 and IL-5, represent two extreme stages of polarization. Th1 cells are effective against intracellular microbes, partly because IFN-γ stimulates antimicrobial mechanisms within infected cells. In contrast, Th2 cells are effective against helminths and blood-borne parasites, partly through the induction of IgE antibody and eosinophils that kill the parasites. The cytokines produced in the local microenvironment modulate the type of response that will be generated. For example, IL-12 induces Th1 cells whereas IL-4 induces Th2 cells. Furthermore, IL-25 appears as a main controller of type 2 responses [26•].

DCs play a critical role in determining the type of induced response. In murine spleen, CD8α+ DCs, which secrete IL-12, induce Th1 responses, whereas CD8α– DCs, which do not secrete IL-12, induce Th2 responses [27,28]. In humans, purified blood mDCs and pDCs can induce Th1 and Th2 responses *in vitro*, respectively [29]. DCs may also be important in maintaining the induced type of immunity. For example, a *Leishmania* antigen (LACK), used as an inducer of allergic airway inflammation, showed that the persistence of LACK-specific Th2 T cells was associated with long-lived LACK-loaded DCs with a phenotype of pDCs [30••]. However, the polarizing effects of DC subsets may be susceptible to microenvironmental signals including microbes and other cells products, as discussed below.

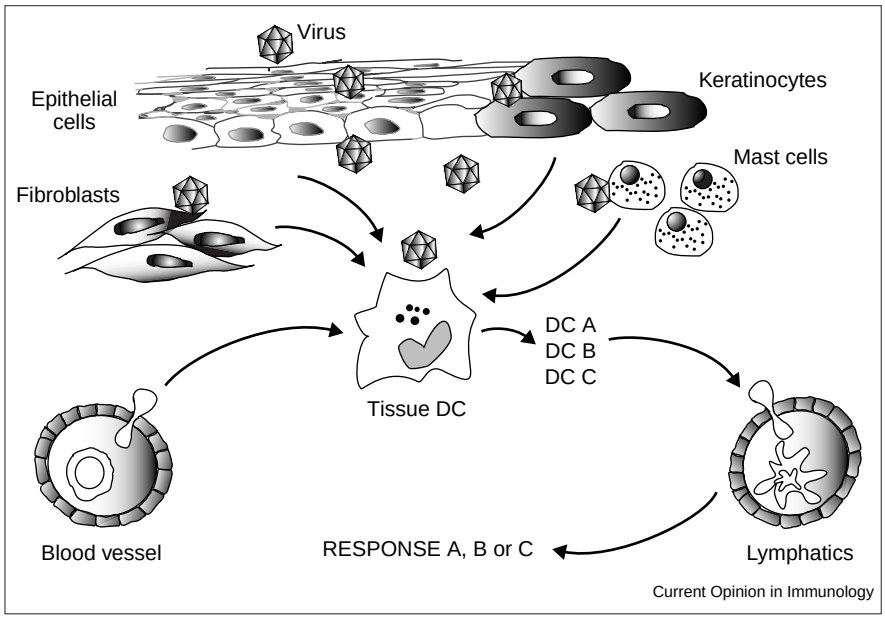
How dendritic cells sense microbes

Immature DCs, present at the site of infection, can sense microbes directly by recognizing molecular patterns within microbial carbohydrates, lipids and nucleic acids using highly conserved pattern-recognition receptors [1•,31]. Such receptors include Toll-like receptors (TLRs) [32•,33•] as well as lectins (e.g. mannose receptor, DEC-205 and DC-SIGN) [34,35••]. In *Drosophila*, different TLRs transduce signals from different microbes to elicit distinct antimicrobial peptides. In mice, *Escherichia coli* lipopolysaccharide (LPS) signals through TLR4, whereas cell wall components of Gram-positive bacteria, and peptidoglycans from *Staphylococcus aureus*, signal through TLR2 [36]. Minor structural differences may lead to the engagement of different TLRs, as illustrated by *E. coli* LPS, which induces a Th1 response via IL-12 secretion, whereas *Porphyromonas gingivalis* LPS, which triggers TLR2, induces a Th2 response [37•]. Distinct DC subsets carry different sets of TLRs. In particular, pDCs, but not mDCs, express Toll9R (receptor for microbial demethylated DNA) and Toll7R (whose natural ligands have not yet been characterized). Conversely, mDCs, but not pDCs, express Toll2R, Toll4R and Toll6R [38•,39•].

Viruses use a large variety of surface molecules for their anchoring and different strains of the same virus may use different receptors to enter their host cells. For example, CD46 was identified as a cellular receptor for the Edmonston strain of measles virus (MV), yet many isolates use SLAM/CD150 [40]. Likewise human CMV (HCMV) fibroblast-adapted strains do not bind DCs, whereas endothelium-tropic strains do and consequently alter DC functions [41••]. Finally, CD4 together with CXCR4 and CCR5 were long considered as primary receptors for HIV.

Figure 2

The impact of the microenvironment of DC maturation on DC function. Following DC migration from blood vessels, microbes (e.g. a virus is shown here) can induce DC maturation directly by hitting pattern-recognition receptors on DCs. Alternatively, other cell types (epithelial cells, keratinocytes, mast cells and fibroblasts) may recognize the danger and secrete cytokines (IL1, TNF-α, IFN, etc.), heat-shock proteins or eicosanoids (PGE_2, leukotrienes, etc.). Such microbe-induced microenvironments may result in DC activation and trigger their migration to lymphoid tissue via lymphatics. However, this microenvironment may also modulate DCs and skew their antigen-presenting and T cell activating capacity. Hence, various responses are possible, depending on the type of DC and the stimuli received by the DC.



However, DC-SIGN, a lectin that is expressed on intDCs, represents a primary HIV anchor [35••,42].

One may imagine that, as a part of their evolutionary trend to survive, microbes have stripped any element that would trigger DC activation. Such a situation could result in uncontrolled microbial replication, which would be deleterious to the host. This may have prompted the evolution of alternative detection mechanisms. Indeed, the presence of the microbe can be sensed indirectly, via neighboring tissue damage as well as activation of cells such as keratinocytes, epithelial cells, fibroblasts and mast cells [31]. The release of inflammatory cytokines such as IL-1β, TNF-α and GM-CSF, or heat-shock proteins from dying cells [43•], creates a microenvironment that activates immature DCs (Figure 2). Another good strategy for a microbe to avoid immunity is to alter the migration of DCs. Poxviruses, for instance, do so through the release of chemokine antagonists [44,45•]. To counteract such strategy, neutrophils release β-defensins (anti-microbial polypeptides) and MIP-3α, both of which are able to attract additional immature DCs to the site of infection [46]. Thus, several molecular pathways alert DCs to the microbe invasion.

How dendritic cells respond to microbes

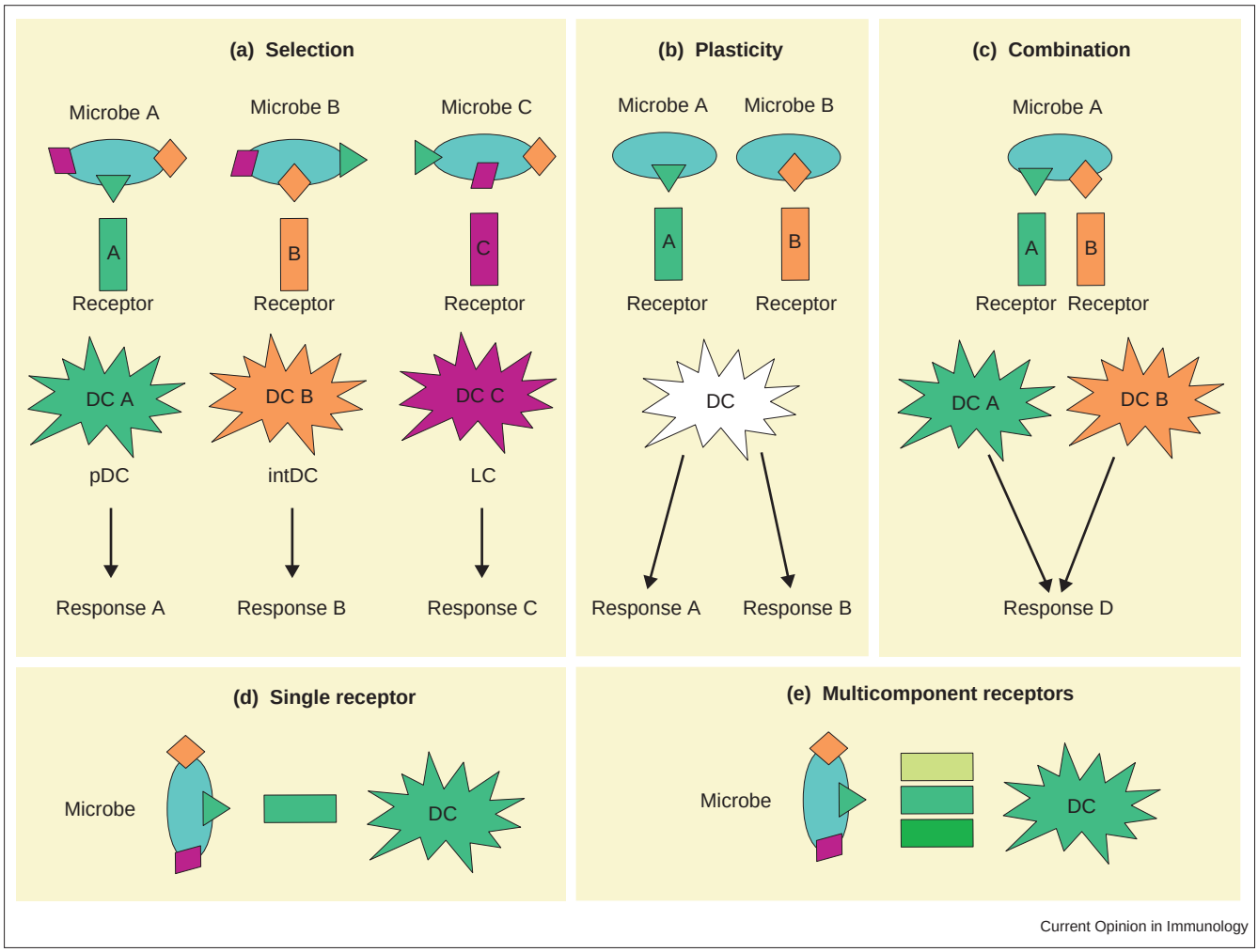
The molecular response of DCs to microbes represents one of the most critical steps in the induction of protective immunity. It is unlikely that a microbe–DC interaction is mediated by a single set of ligand–receptor interactions. Rather, DCs express repertoires of pathogen-recognition receptors that are able to recognize molecular patterns expressed by microbes (Figure 3). Indeed, DC maturation induced by whole bacteria is consistent with the induction of several pathways [47•,48•].

Several models can be envisioned to understand the induction of protective immunity (Figure 3). Thus, in a selection model, different microbes can target distinct DC subsets through a unique set of receptors, for example Langerin on LCs, DC-SIGN on intDCs or BDCA-2 on pDCs [49•]. However, there is equally compelling experimental evidence for plasticity, where different microbes, or different forms of the same microbe acting through different receptors on the same cell, modulate DCs to induce different responses. For example, the yeast form of the fungus *Candida albicans* induces a Th1 response via induction of IL-12 in DCs, whereas its hyphal form inhibits IL-12 and stimulates IL-4 production by the DCs themselves [50]. Similar principles have been described for *Aspergillus* infection [51•].

Microbe-induced microenvironments can influence DC function also through indirect mechanisms, such as inflammatory molecules or toxins. For instance, prostaglandin PGE-2 modulates DCs to induce Th2 responses (reviewed in [52]). Similarly, microbial toxins (e.g. from *Vibrio cholerae*) can modulate otherwise Th1-inducing DCs to promote Th2 responses [53]. Furthermore, viruses stimulate pDCs to secrete IFN-α and induce their differentiation into DCs that prime IFN-γ- and IL-10-producing T cells [54]. Alternatively, IL-3, often present in high amounts at the site of parasite invasion, induces pDCs to differentiate into Th2-inducing DCs [29].

Furthermore, a microbe may target two distinct DC subsets, leading to a mixed response through a combination of signals (Figure 3c). For instance, recent studies show that dengue virus targets two DC subsets, monocyte-derived DCs (resembling intDCs) and human skin LCs [55].

Figure 3



The molecular response of DCs to microbes. Several models can be envisioned: **(a)** selection, where different microbes target distinct DC subsets (e.g. here, DCs A, B and C are pDCs, intDCs and LCs, respectively) through sets of receptors that are unique to each DC subset; **(b)** plasticity, where different microbes, or different forms of the same microbe acting through different receptors on the same cell, modulate DCs to induce different responses; and **(c)** a microbe may target two distinct DC subsets, leading to a mixed response through a combination of signals. **(d)** It is unlikely that a microbe–DC interaction is mediated by a single set of ligand–receptor interactions. **(e)** Rather, DCs express repertoires of pathogen-recognition receptors that are able to recognize molecular patterns expressed by microbes.

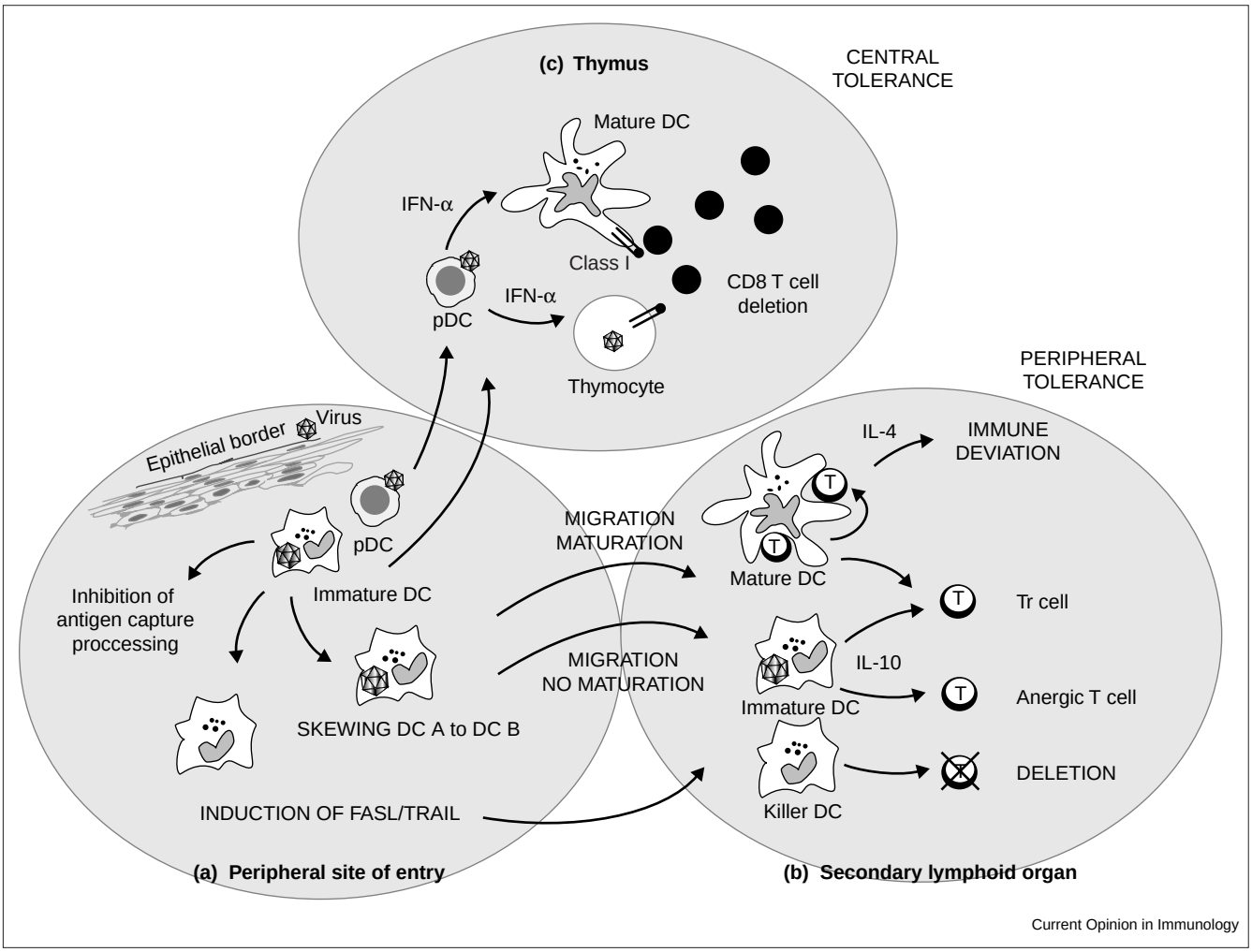
Finally, another scenario involves the interplay between distinct DC subsets. For example, in response to viral triggering pDCs release high amounts of IFN-α. This IFN-α induces monocytes to differentiate into DCs with high antigen-capture and -presentation capacity, leading to the amplification of immune responses [56,57••]. The immunomodulatory properties of IFN-α have long been recognized. However, it was only recently shown that its adjuvant effect is mediated through the activation of DCs [58••]. In particular, complete Freund's adjuvant, which contains killed *Mycobacteria*, is inefficient in IFN-receptor$^{-/-}$ mice [58••].

In vivo studies on the role of DCs in triggering antimicrobial immunity are relatively few. It has been shown, however, that *Leishmania* can stay for protracted periods of time within DCs thereby maintaining antigen-specific immune responses and protecting the mice from reinfection. Virulent *Salmonella typhimurium* differentially targets splenic DC subsets in mice upon oral infection, determining their tissue distribution, numbers and cytokine profile [59•]. Furthermore, DCs capture and retain BCG upon *in vivo* infection. In the early stages of infection, the DCs present the BCG antigens but this property is lost at later stages, suggesting that BCG may use DCs to hide from the immune system [60•].

How microbes evade dendritic cells

Microbes have established numerous strategies to survive and to evade antimicrobial strategies of the host [45•,61,62]. They can interfere at several steps of DC-induced immunity, as detailed below and in Figure 4:

Figure 4



Tolerance mechanisms used by microbes (e.g. a virus is shown here) to prevent establishment of microbe-specific immunity. (a) At the peripheral site of entry, microbes may: inhibit antigen capture and processing; skew DC phenotype and function; or kill DCs. Microbes may also prevent DC migration and/or maturation and alter DC cytokine secretion. (b) This would contribute to peripheral tolerance in secondary lymphoid organs by: causing immune deviation to Th2 response via IL-4; prevention of DC maturation may generate anergic T cells or Tr cells; alternatively, the maturation of killer DCs expressing FasL/TRAIL can induce deletion of specific T cells. (c) Central tolerance (in the thymus) may also be used by microbes to avoid immunity. For example, HIV-induced release of IFN-α by thymic pDCs results in increased MHC class I expression and possibly costimulatory molecules on mature DCs and thymocytes, which will lead to the deletion of specific thymocytes.

these include, firstly, DC generation, survival and maturation; secondly, antigen processing/presentation; and thirdly, T cell activation/priming. Among the best-studied pathogens targeting DCs are HIV [35••,63], MV [64], LCMV [65••], herpes simplex virus 1 (HSV-1) [66,67], CMV [41••] and *Salmonella* [59•].

Interference with generation and survival of dendritic cells

Microbes can affect DC recruitment to tissue, their differentiation and their survival. For instance, poxviruses and herpesviruses encode secreted homologs of chemokine receptors that act as chemokine antagonists to prevent the attraction of additional DCs at infection sites [45•,68]. Human T cell leukemia virus type 1 can infect monocytes and impair their differentiation into DCs. Canarypox and vaccinia viruses inhibit DC maturation and induce DC death by triggering apoptosis [69]. Viruses like MV [64] or HIV [70] induce DCs to form syncytia in which the virus can replicate. Several bacteria can also affect DC viability. In particular, *Shigella* and *Salmonella* deliver into the DC cytoplasm molecules that activate the proapoptotic machinery, such as caspase 1 [71]. Evolution has found, however, a countermeasure to these microbial attacks in the form of cross-priming and cross-presentation. Under these mechanisms, the remains of microbe-killed DCs are phagocytosed by viable DCs, which can now process and present the microbial antigens [72,73••,74].

Interference with antigen-presenting mechanisms

Microbes have evolved several means to interfere with the ability of DCs to present antigens to T cells thereby

leading to interference in the induction of immune responses. For instance, microbes can prevent DC maturation either directly as is the case with HSV-1 and vaccinia virus [69] or indirectly as is the case with *Plasmodium-falciparum*-infested erythrocytes [75].

Inhibition happens through various means: these include, firstly, the secretion of altered receptors that block cytokines such as type I interferon, IL-1 and TNF-α, which are potent DC maturation factors; secondly, the secretion of regulatory cytokines such as the viral IL-10s from Epstein–Barr virus (EBV) or CMV; thirdly, the inactivation of intracellular pathways such as those targeted by HCMV to prevent surface expression of costimulatory molecules and MHC–peptide complexes; or fourthly, the blocking of expression of chemokine receptors, such as CCR7, which results in the inability of DCs to migrate to the draining lymphoid organ (reviewed in [45•]).

Inhibition of DC maturation has two beneficial consequences for microbes: firstly, prevention of microbe-specific immunity (i.e. immune ignorance); and secondly — even more deviously — induction of microbe-specific tolerance, when immature DCs present microbial antigens in the absence of costimulatory signals. This may indeed represent one of the early mechanisms used by HIV to escape immune responses [6•].

The bacterium *Bordetella pertussis* — the whooping-cough agent — has also developed a strategy to generate antigen-specific T regulatory cells (Tr cells) to evade protective Th1 responses [76••]. During acute infection with *B. pertussis*, Tr cells specific for *B. pertussis* filamentous hemagglutinin (FHA) and pertactin are generated at the mucosal surfaces. The Tr cells secrete IL-10 but neither IL-4 nor IFN-γ and are able to suppress the Th1 responses against *B. pertussis*, as well as unrelated pathogens, thus explaining the immunosuppression that is induced by *B. pertussis*. The generation of Tr cells is mediated by FHA, which inhibits DC IL-12 secretion but promotes their secretion of IL-10 [76••,77,78•].

Interference with T cell activation

Viruses can modulate cytokine release by DCs (reviewed in [45•]). For instance, DCs infected with MV or HCMV activate T cells but are poorly efficient at inducing/sustaining their proliferation. This defect may be due to inhibition of factors contributing to T cell proliferation and differentiation, like inhibition of IL-12 secretion by MV and Rauscher leukemia virus (RLV) [79]. This inhibition of cytokine secretion may be due to a total block of secretion or alternatively to a skewing of the pattern of secreted cytokines. In particular, RLV not only blocks IL-12 but also induces DCs to secrete IL-4, which results in immune deviation, one of the forms of tolerance induction [79]. The inhibition of T cell proliferation may also partly be due to the fact that these virally infected DCs become killer cells [41••,80•]. For example, MV and HCMV render DCs cytotoxic through the upregulation of both FasL/CD95L and TRAIL on DCs. The virus sensitizes the activated T cells, which are otherwise resistant to these molecules' pro-apoptotic effects [41••]. Endogenous IFN-α may be responsible for virus-induced upregulation of TRAIL as previously shown on DCs [81].

How microbes subvert dendritic cells

In their infinite struggle for evolutionary survival, microbes have learned how to utilize DCs for their own benefits. In the gastrointestinal tract, entry of pathogens occurs mainly through M cells — specialized cells concentrated in the epithelium overlying the Payer's patches. Although M cells are highly selective and do not allow entry of all microbes, their unique glycosylation and adhesion-molecule patterns can be utilized by some microbes. For instance, *S. typhimurium* can trigger massive cytoskeletal rearrangement of M cells, promoting its engulfment. The pathogens that cross the gut epithelial barrier through the M cells directly encounter DCs and macrophages, which accumulate in intraepithelial pockets under M cells [82]. DCs themselves may be utilized by pathogens to cross the gut epithelial barrier through an intriguing anatomical feature, as these cells scan the content of the intestinal tract by sending dendrites, like periscopes, into the lumen. To preserve the integrity of the mucosal barrier, DCs express tight-junction proteins and form new tight-junction-like structures with neighboring epithelial cells [83•]. Whether this mechanism is constitutive or induced in response to microbial signals is unclear.

Given the dramatic impact of HIV on mankind, much effort has been spent on the relationship of HIV and DCs, which has led to the discovery of several novel mechanisms. As mentioned above, HIV binds with high affinity to a DC-specific lectin called DC-SIGN. The virus — at least subtype B, which is the most prevalent in the Western World — does not seem to replicate there, but rather uses the DC for a free ride into the draining lymphoid organ. As DC-SIGN also mediates clustering of DCs with T lymphocytes — a fundamental event in the initiation of the immune response — it would be advantageous for HIV to increase DC-SIGN expression. That indeed is the case and, as shown recently, HIV Nef protein inhibits DC-SIGN endocytosis, leading to dramatically increased surface expression of DC-SIGN, thus facilitating virus transmission to T cells [84•,85••]. HIV may also act by inducing central tolerance against its own components through deletion in the thymus of HIV-specific T cells (Figure 4c). Indeed, a recent study shows that HIV-1 upregulates MHC class I on infected as well as uninfected thymocytes through IFN-α, which is released by HIV-infected pDCs [86•].

DCs are also the targets of microbial pathogens spread by insects and arthropods. For example, Venezuelan equine encephalitis virus (VEE) targets LCs, which serve as replication sites and transport the virus into the draining lymph node [87]. Inevitably, DCs are also the target of prions,

which cause the orally transmissible bovine spongiform encephalopathy (BSE or mad-cow disease), its human variant Creutzfeld–Jacob disease, and possibly sheep scrapie [88•,89]. Follicular DCs (FDCs) — cells of mesenchymal origin restricted to primary lymphoid follicles as well as germinal centers of secondary follicles — are essential for the replication of prions before they spread into the nervous system [90]. There is now evidence that splenic mDCs carry large amounts of prion proteins [89] and that DCs infected with prions *in vitro* can transport intestinally administered prions directly into lymphoid tissues *in vivo* [88•].

Harnessing dendritic cells for increased antimicrobial immunity

The study of the interactions of microbes with the immune system in general, and DCs in particular, has also led scientists to subvert the microbial stratagems, in order to improve immunity. *In vivo* induction of microbe-specific immune responses was first demonstrated in animals injected with DCs loaded with BCG. Studies showing that injection of pathogen-loaded DCs can lead to the development of protective immunity followed quickly in models of infectious disease, such as *Borrelia burgdorferi*, which is the agent that causes Lyme disease [91], *Chlamydia trachomatis*, which causes venereal disease and blindness [92], and *Mycobacterium tuberculosis* [93].

In most cases, DCs were loaded with either killed microbes or their components. Care should, however, be taken, because DCs pulsed with microbial components, for example chlamydial major outer membrane protein antigen, elicit a non-protective type 2 immune response, as opposed to DCs loaded with the whole inactivated organism, which elicit a protective type 1 immune response [94]. Alternative means of antigen delivery to DCs are also being tested. For instance, on the basis of studies performed in the context of tumor immunity, DCs pulsed with fungal RNA were shown to protect against *C. albicans* in healthy animals as well as animals recovering from allogeneic bone marrow transplantation, a clinically relevant situation [95•]. Studies using bacteria-loaded DCs have recently brought new paradigms for the development of immune responses. For example, DCs loaded with *Streptococcus pneumonia* induce antibody production in a T-cell-dependent manner, although this response was long considered to be T-cell-independent [96•].

The study of microbes and their products has led to the development of novel approaches to immunotherapy. Bacteria and viruses have been extensively used as vaccine carriers to induce or boost protective immune responses. Efforts are being made at improving targeting of DCs with these vectors. In particular, engineered versions of *Salmonella*, *Shigella* and *Listeria* have been prepared as oral carriers for genetic immunization that preferentially targets DCs. Such vectors have been effectively used for treatment of mice carrying tumors [97,98].

Cholera toxin is the most powerful mucosal adjuvant and its subunit B appears to be a candidate to induce strong type 2 responses [99]. Other microbial products are able to turn-on cellular immune responses. In particular, the *Klebsiella pneumoniae* outer membrane protein A (OmpA), which binds to TLR2 [100], and the B subunit of Shiga toxin [101] permit the delivery of antigens into the MHC class I presentation pathway and induce protective antitumor responses. Microbes have developed proteins, among others HIV Tat and HSV VP22, containing motifs called protein transduction domains (PTDs) that are capable of transducing cargo across the plasma membrane, allowing the protein to accumulate within the cell [102]. Tat–OVA, a fusion protein of the 11-amino-acid Tat PTD with OVA, has been used to transduce DCs and yield MHC class I and II peptides [103]. Administration of these transduced DCs to animals leads to regression of OVA-expressing tumors.

Finally, antigen-loaded DCs can be used to elicit antiviral immune responses. This has been now demonstrated in humans both *in vitro* for influenza virus, EBV [104] and HIV [105], and *in vivo* upon injection of peptide-pulsed DCs in healthy volunteers and patients with advanced cancer [106•,107].

Conclusions

Vaccines against microbial agents clearly represent a success story of immunology and have saved thousands of people from smallpox, polio, measles, tetanus and hepatitis. Yet, infectious diseases still represent a main health problem. AIDS, hepatitis C and malaria as well as biological agents that could be deliberately spread remain a major challenge for immunologists. Our progress in the understanding of DC physiology will hopefully help us create the necessary vaccines to counteract these scourges.

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