

Dendritic Cells: Translating Innate to Adaptive Immunity

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Abstract The innate immune system provides many ways to quickly resist infection. The two best-studied defenses in dendritic cells (DCs) are the production of protective cytokines—like interleukin (IL)-12 and type I interferons—and the activation and expansion of innate lymphocytes. IL-12 and type I interferons influence distinct steps in the adaptive immune response of lymphocytes, including the polarization of T-helper type 1 (Th1) CD4⁺ T cells, the development of cytolytic T cells and memory, and the antibody response. DCs have many other innate features that do not by themselves provide innate resistance but are critical for the induction of adaptive immunity. We have emphasized three intricate and innate properties of DCs that account for their sentinel and sensor roles in the immune system: (1) special mechanisms for antigen capture and processing, (2) the capacity to migrate to defined sites in lymphoid organs, especially the T cell areas, to initiate immunity, and (3) their rapid differentiation or maturation in response to a variety of stimuli ranging from Toll-like receptor (TLR) ligands to many other nonmicrobial factors such as cytokines, innate lymphocytes, and immune complexes. The combination of innate defenses and innate physiological properties allows DCs to serve as a major link between innate and adaptive immunity. DCs and their subsets contribute to many subjects that are ripe for study including memory, B cell responses, mucosal immunity, tolerance, and vaccine design. DC biology should continue to be helpful in understanding pathogenesis and protection in the setting of prevalent clinical problems.

1 Innate Defenses Provided by DCs

1.1

Introduction

The innate and adaptive arms of the immune system deal with a remarkable diversity of microbial agents. Many defense mechanisms have evolved. Some of the innate defenses (Table 1) are known to be well developed in dendritic cells (DCs), particularly the production of immune-enhancing cytokines and the mobilization of innate lymphocytes (NK, NKT, $\gamma\delta$ T). In addition, DCs have a number of innate or built in properties that lead to strong, adaptive immunity including memory. DCs are sentinels. They are able to capture and process antigens effectively and to migrate to lymphoid tissues, where the immune system is alerted to make an appropriate response. At the same time, DCs are sensors, undergoing extensive, stimulus-dependent and typically irreversible differentiation, which is called “maturation.” The maturation stimulus influences the type of the ensuing immune response, e.g., T-helper type 1 (Th1) vs Th2 types of CD4⁺ T cell responses [1–3]. DCs undergo maturation in response to microbial and nonmicrobial stimuli and initiate adaptive immunity not only to infection but also to transplants, tumors, autoantigens, and allergens. Following a brief summary of the innate defenses that are provided by DCs, we will concentrate on the properties that allow these cells to link innate and adaptive immunity, particularly antigen-specific, T cell-mediated immunity.

Table 1 Mechanisms of innate resistance to infection

Phagocytosis
Secretion of proteins and peptides
Microbial binding lectins and pentraxins
Defensins and other antimicrobial peptides
Complement
Interferons and other cytokines
Innate lymphocytes

1.2

Definition

The term “innate immunity” has two equally rich meanings. One refers to innate “defenses,” processes that provide rapid resistance to microbial infection (Table 1). There are many such defenses, but as a group they do not have the

degree of specificity and the memory that are characteristic of adaptive resistance. Innate defenses are expressed by many different bone marrow-derived cells and in some cases by non-hematopoietic ones. A second meaning of innate immunity is “built in.” These are properties that often do not directly contain a pathogen but instead allow for the transition to adaptive immunity. Several innate features are most developed in DCs including antigen capture and presentation, homing to lymphoid tissues, and maturation in response to a plethora of stimuli. These features of DCs allow an early response to be translated into adaptive resistance and memory.

1.3

Phagocytosis

The classical innate defense mechanism is phagocytosis, followed by intracellular killing and digestion. This is the form of host resistance discovered by Metchnikoff, the father of innate immunity. Granulocytes and macrophages are the principal phagocytes. DCs have phagocytic properties but, typically, uptake is not extensive and limited to one or a few particles per cell. Moreover, phagocytosis by DCs is not yet known to contribute to microbial clearance and killing, i.e., innate defenses, although it is clearly valuable for efficient antigen processing and presentation on MHC class I and II products [4, 5].

1.4

Cytokines

Several innate defenses involve the secretion of proteins and peptides (Table 1). For DCs, cytokines have been the main products studied to date. For example, DCs are known to produce large amounts of interleukin (IL)-12 and type I interferons. In some instances where it has been studied, DCs are the major source of these immune-enhancing cytokines upon microbial exposure *in vivo* [6, 7]. These cytokines provide innate resistance, e.g., IL-12 mobilizes natural killer (NK) cells while interferons are anti-viral. In addition, innate lymphocytes and interferons act back on the DCs to drive maturation, and on T and B lymphocytes to enhance adaptive immunity (reviewed in [8, 9]).

1.5

Innate Lymphocytes

DCs are distinct in being able to expand the numbers and function of different types of innate lymphocytes, and then to respond through maturation to induce adaptive immunity. Innate lymphocytes are able to produce large amounts of protective cytokines within hours of stimulation, particularly

type II or γ -interferon, but the lymphocytes do not seem to develop memory. NK cells are the prototype [10–12], but NKT cells [13, 14] and $\gamma\delta$ T cells [15] might be included. The interaction of DCs with these innate lymphocyte leads to tumor necrosis factor (TNF)- α and IL-12 production by the DCs, as well as further DC differentiation or maturation to elicit adaptive immunity to captured antigens. This topic has been reviewed elsewhere [16], including the fact that some innate lymphocytes are able to sense both infected and transformed or “stressed” cells.

1.6

Summary

DCs exhibit innate responses that can lead quickly to resistance or defense against infection. The two best-characterized responses are the production of large amounts of immune-enhancing cytokines and the mobilization of innate lymphocytes. However, as we shall now discuss at length, DCs also have innate properties that allow for the induction of adaptive immunity. Three sets of innate features are vital to the initiation of adaptive immunity. These are: (1) the distribution of DCs in vivo as sentinels for antigen capture and clonal selection of T cells, (2) the repertoire of antigen receptors and processing capacities expressed by DCs, and (3) the ability of DCs to sense microbial and other stimuli to undergo maturation.

2

The Tissue Distribution and Migration of DCs In Vivo

2.1

Definition

DCs were discovered as a distinct type of leukocyte with distinct functions, particularly potent stimulation for responses by naïve and resting T lymphocytes. The DCs could be identified and enriched based on several properties: their expression of high levels of antigen-presenting MHC class II molecules (originally termed “Ia antigens” [17, 18]), a lack of typical properties of macrophages and lymphocytes, a distinct morphology including a stellate cell shape and unusual motility involving the continual extension of cell processes in many directions, and the 33D1 [19, 20] and NLDC-145 antigens [21]. Many of these properties were used to identify DCs in different tissues [22–26], including their localization in tissue sections [27]. DCs were found at body surfaces, in the interstitial spaces of most organs except the brain parenchyma, in afferent lymph, and in the T cell areas of spleen, lymph

nodes, and Peyer's patch. The identification of DCs in lymph [28–32] was critical to the idea that DCs could leave peripheral organs and migrate to the T cell areas to initiate cell-mediated immunity. The migration of DCs has recently been reviewed [33].

2.2

DCs at Body Surfaces

The translation of innate to adaptive immunity requires that antigens, which are typically deposited in peripheral tissues during infection, gain access to lymphoid organs. The latter are designed to facilitate the selection of rare clones of antigen-reactive lymphocytes from the recirculating pool. DCs fulfill this requirement, serving as sentinels to pick up antigens and then move to the lymphoid organs to initiate immunity. DCs form networks of cells along body surfaces, often intimately associated with the epithelium. In skin, the DCs or Langerhans cells are found in the suprabasal layer [34]. In respiratory epithelium, DCs are found within and just below the epithelium [35, 36]. In the intestine, DCs lie just below the epithelium [37], but in some instances—as in the ileum—they send processes between the occluding junctions into the lumen [38, 39]. In mucosal-associated lymphoid organs, like the Peyer's patch, but also oral-associated lymphoid organs like the tonsils and nasal-associated lymphoid tissue, DCs lie beneath a specialized epithelium containing antigen-transporting M cells [40–42]. A new network of DCs has just been identified in the muscularis layer of the mouse intestine [43]. In order for DCs to home to inflamed epithelia, an important interaction is the chemokine CCL20/MIP-1 α made by the epithelium and the chemokine receptor CCR6 on the DCs [40, 44]. Fractalkine and CXCR3 additionally are responsible for the extension of DC processes through epithelia [38]. In the steady state, i.e., in the ostensible absence of infection and inflammation, peripheral DCs continuously capture environmental proteins, e.g., from the airway and intestinal lumen [45, 46], as well as self constituents [47]. In sum, DCs are positioned for antigen capture, but what is interesting is that this seems to go on during the steady state, a feature that may allow DCs to induce tolerance to harmless self and environmental antigens, as we will stress (Sect. 6.4).

2.3

DCs in Afferent Lymph

When afferent lymphatic vessels are cannulated, DCs are always found in the effluent, so that DCs seem to be migrating continuously in the lymph, from tissues to lymphoid organs [48]. The protein within the lymph then leaves the

lymphoid tissue in efferent lymphatics to enter the thoracic duct and return to the blood stream, whereas the DCs remain within the local lymphoid organ and are typically not found in efferent or thoracic duct lymph. Even in the steady state, DCs are carrying cargo from the periphery. For example, in the mesenteric lymph, a subset of DCs contain apoptotic bodies, earlier termed DNA positive inclusions [31]; these cells also can be marked for the keratins and nonspecific esterases of the intestinal epithelium [47]. The DCs in the lymph in the steady state may arise from DCs that are trafficking from the blood into tissues prior to entry into the lymph, but a subset of monocytes may also be a source [49–51]. DC migration into the afferent lymph can also be increased markedly in response to many different stimuli. This likely involves an increased expression of CCR7 on the DCs [52] as well as the corresponding chemokines, CCL19 and 21 on lymphatics and in the lymph node [53, 54]. Recently, Pasare and Medzhitov used lipopolysaccharide to stimulate DC migration during protein immunization [55]. They found that a lack of the Toll-like receptor (TLR) adaptor protein MyD88 blocked immunization but did not alter the increase in DC migration to the lymph nodes in response to lipopolysaccharide (LPS), indicating that these components of DC function—immunization and migration—are separately controlled [55].

2.4

DCs in Blood

DCs have been studied in blood from humans and monkeys, where they comprise at least two subsets termed myeloid and plasmacytoid (PDCs) [56], expressing high and low levels of the CD11c integrin in humans. In mice, PDCs do express some CD11c. Myeloid and PDCs are distinguished as well on the basis of commercially available antibodies to blood DC antigens or “BDCAs,” e.g., BDCA-1 for myeloid DCs and BDCA-2 for PDCs. Circulating myeloid DCs and PDCs may derive directly from the marrow in the steady state, and these outputs can increase in infection [57, 58]. During perturbation it is additionally possible that DCs can be mobilized from tissues and move into the blood and then to the spleen. The latter seems to occur during transplantation [59] and would allow DCs from the graft to stimulate the direct pathway of rejection, as occurs in the primary mixed leukocyte reaction, a classical assay for the immunostimulating function of DCs [60].

2.5

DCs in the T Cell Areas of Peripheral Lymphoid Tissues

Once in the lymphoid organ, most DCs are found in the T cell areas. Traffic to the B cell areas may well occur but needs further definition. It is often

assumed that DCs in the T cell areas are continually derived from the lymph, but precursors to some subsets of DCs may enter from the blood. Also, proliferating DCs and DC precursors have been identified in mouse spleen [61] and skin [62], allowing for local regeneration and expansion of DC numbers. Identification of DCs in tissue sections is achieved by a combination of criteria in addition to their large irregular cell shape. These include high expression of MHC class II and the CD11c integrin, a lack of lymphocyte and macrophage markers, and expression of various receptors for antigen uptake (see Sect. 3). The distribution of DCs in the lymph node has now been visualized by two-photon microscopy of living tissue. Migrating mature DCs arrive in the T cell area where they efficiently select T cells specific for the presented antigen [63–65]. In the T cell area, these DCs join a network that is present in the steady state [66]. Stable contacts develop when antigen-bearing DCs encounter their cognate T cells, and these contacts persist for a day or more, both in the steady state when DCs can be tolerogenic, or upon maturation when immunity develops [67, 68]. This fits the observation that the commitment of T cells to proliferation, which can occur prior to either tolerance or immunity [69–71], requires sustained triggering by antigen or mitogen for about a day [72]. In the steady state, one also can observe some DCs in the subcapsular sinus, quite distinct from the highly endocytic and more numerous macrophages there [66]. These DCs seem to be in transit from lymph to the deeper T cell areas. In contrast, the network of DCs in the T cell area continually form and retract processes but do not move translationally [66], much as was observed *ex vivo* when DCs were discovered more than 30 years ago [73]. In sum, while DCs constitute just a few percent of the cells in a lymph node, their size and pervasive cell shape puts them in a position to scan T cells circulating through lymphoid tissues and then to select clones appropriate for the presented antigens.

2.6

DCs at Mucosal Surfaces and in Mucosal-Associated Lymphoid Tissues

As mentioned, DCs are positioned for antigen capture at internal or mucosal body surfaces. Their subsequent traffic can include movement to the draining lymph nodes, e.g., the mesenteric node in the case of the intestine, as well as movement to the T cell areas (“interfollicular zones”) of the mucosal-associated lymphoid tissues, like the Peyer’s patch. It is possible that DCs in the epithelium and lamina propria home to the mesenteric lymph node, whereas DCs beneath the antigen-transporting epithelium of the Peyer’s patch home to the local T cell area. Injection of TNF- α or TLR ligands leads to a marked mobilization of DCs from the intestine into the mesenteric afferent

lymph [74]. DCs at mucosal surfaces are likely to be important for studying the generation of protective immunity as well as the maintenance of tolerance against chronic inflammatory disease and allergy.

2.7

Summary

A significant innate property of DCs, which facilitates their function in the initiation of adaptive immunity, is their ready access to antigens and their capacity to move to the peripheral lymphoid tissues. Antigen uptake can be enhanced by numerous potential endocytosis receptors (next section), while migration to the T cell areas facilitates the selection of T cell clones and determines T cell fate decisions involved in peripheral tolerance and immunity. These features of DCs are deployed in the steady state, where they can be used to induce peripheral tolerance to self and harmless environmental antigens (reviewed in [75]). There are still substantial gaps in our knowledge of several topics, e.g., the control mechanisms for DC migration and homing in the steady state, movement into the B cell areas, and traffic at mucosal surfaces.

3

Antigen Uptake Receptors and the Endocytic System of DCs

3.1

Definition

The translation of innate to adaptive immunity requires that antigens be captured and processed intracellularly prior to the formation of ligands (“presented antigen”) for the T cell receptor. Generally these ligands are complexes of peptides with MHC products, but others are being identified, such as complexes of glycolipids with CD1 molecules. The term antigen presentation is best restricted to the uptake, processing, and presentation of processed antigens, e.g., as peptide–MHC complexes. All cells with MHC products present antigens, but as we will summarize here, DCs are specialized in each of the uptake, processing, and presentation steps. In subsequent sections, we will consider the additional accessory or costimulating functions of DCs that by definition work together with presented antigen to stimulate adaptive immunity.

3.2

A Spectrum of Potential Antigen Receptors on DCs

Early studies with cells from mice [76–79] indicated that many DCs were in an immature state and needed to acquire their immune-inducing capacities. In

some instances this only required a short period in culture. The term “immature” described the lack of strong T cell-stimulating activity, but the capture of antigens is often selectively expressed by immature forms of DCs [80, 81]. Immature DCs express an array of receptors that are able to mediate endocytosis (Fig. 1). Uptake function has been studied in three ways. In some cases ligands have been followed into the cell. In others, antibodies to the lectin have been used as surrogate ligands to document uptake. Third, endocytosis has been predicted by characteristic coated pit localization sequences in the cytosolic domains and additional motifs for targeting within the cell.

Many uptake receptors on DCs, and the list seems to be expanding, are calcium dependent or C-type lectins (Fig. 1). These may form multimers but not by covalent means, in contrast to the numerous dimeric C-type lectins that are expressed on NK and other cells. C-type lectins on DCs can either be type II transmembrane proteins with a single, carboxy terminal lectin domain, or type I proteins with multiple lectin domains. The sugar recognition properties of some of these lectins have been defined, but in most instances, relatively little is known about the natural ligands for these lectins. DC-SIGN/CD209 is a well-studied exception, since it is able to recognize mannose and fucosyl residues on the surface of a variety of pathogens including human immunodeficiency virus (HIV), cytomegalovirus (CMV), Ebola virus, dengue virus, *Candida*, and certain *Leishmania* [82], while the macrophage mannose receptor/CD206 recognizes mannose residues on certain self constituents, lysosomal hydrolases [83]. DCs also express several potential receptors for dying cells, although these are still poorly defined *in vivo* (Sect. 3.5). Additionally, DCs express Fc γ Rs (and Fc ϵ Rs), which mediate presentation of immune complexes and antibody coated tumor cells on both MHC class I and II. Fc γ Rs also influence the state of DC maturation. Immunoreceptor tyrosine-based acti-

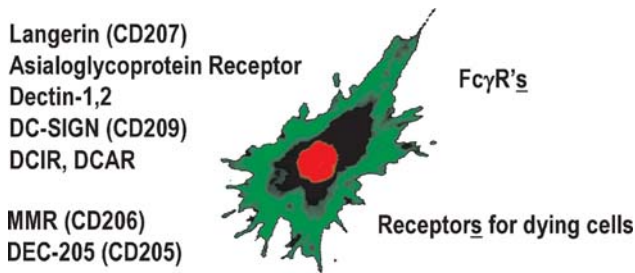


Fig. 1 Potential receptors for antigen uptake by dendritic cells (DCs). Some of the lectins that are expressed by DCs, or DC subsets, are shown. There are several Fc γ receptors for uptake of immune complexes and receptors for dying cells. However, relatively little information on DC receptors for dying cells is available *in vivo*

vation motif (ITAM)-associated receptors stimulate maturation, while Fc γ Rs with inhibitory immunoreceptor tyrosine-based inhibitory motif (ITIM) sequences block it [84, 85]. For most of the potential uptake receptors on DCs, much of the research has been on isolated cells and not in vivo.

3.3

Functions of Endocytic Receptors

There is considerable potential to receptor function beyond ligand binding and uptake (Table 2). We will cite four examples. First, receptors can associate with other signaling molecules. Dectin-1, which is expressed on macrophages and DCs, binds yeast or zymosan particles but, in addition, dectin-1 associates with TLR-2 and thereby signals TNF- α and IL-12 production [86, 87]. Dectin-1 also has an ITAM motif that, following phosphorylation, attracts the src kinase, syk, and mediates production of two other cytokines, IL-2 and IL-10 [88]. Second, receptors can follow distinct intracellular trafficking pathways dictated by sequences in the cytosolic domain. For DEC-205/CD205, a stretch of three acidic amino acids, allows this receptor (uniquely at this time) to slowly recycle through MHC class II positive late endosomal compartments [89]. The MMR/CD206, in contrast, behaves in a more typical way for a coated pit localized receptor, i.e., it enters the cell and rapidly recycles through early endosomal compartments. The trafficking of DEC-205 through MHC II compartments may explain its greatly increased capacity for class II presentation relative to the macrophage mannose receptor (MMR) [89]. A third enigmatic consequence of antigen uptake is cross presentation on MHC class I by DCs, which is evident for proteins captured within dying cells, immune complexes, and DEC-205-associated antigens. DCs are a major cell type for cross presentation in vivo [5, 70, 90–92], but it is not clear how this cross presentation comes about. One proposal is that fusion of the endocytic vesicle with the rough endoplasmic reticulum (ER) is required [93,

Table 2 The endocytic system of dendritic cells

Many potential uptake receptors, sometimes expressed in a DC subset-restricted fashion

Uptake receptors can associate with other signaling molecules like TLRs.

Uptake receptors can route to distinct antigen-processing compartments.

Regulation at many levels, e.g., uptake, intravacuolar pH, protease content

Processing of antigens towards the formation of ligands for T cell receptors, e.g., MHC-peptide, seems efficient, occurring with relatively low doses of administered antigen.

94], but this pathway has been called into question [95]. For some proteins, cross presentation onto MHC class I is clearly dependent on transporters of antigenic peptides (TAP), implying that proteins or their fragments exit the endocytic system for potential proteolysis and TAP transport. A conserved tyrosine in the cytosolic domain of MHC class I molecules may be required for the exogenous pathway, by controlling traffic to a special intracellular compartment [96]. An important part of the cross presentation equation is that in mice, the CD8 α^+ subset of DCs is the principal cross-presenting cell in spleen and lymph nodes [5, 91, 97]. This probably reflects two functions: the capacity of CD8 α^+ DCs to internalize certain ligands, like dying cells and anti-DEC-205 associated antigens [5, 70], and also to efficiently cross-present the internalized ligands following uptake [97, 98]. Fourth, distinct receptors can be expressed on distinct subsets of DCs, which in turn may influence the consequence of antigen uptake and processing (Sect. 3.6). Altogether, the presence of numerous uptake receptors enables DCs to efficiently take up many different ligands, but also, the receptors mediate distinct “post uptake” outcomes.

3.4

The Endocytic System of DCs and Its Regulation

The endocytic system, and not just the repertoire of uptake receptors (Fig. 1), is proving to be a distinctive innate feature of DCs (Table 2). At this point, much of the research involves studies of DCs generated from mouse bone marrow progenitors, or from human monocytes, i.e., there is relatively little information on the bulk of the DCs that occupy lymph and lymphoid tissues. What is emerging from the *ex vivo* studies is a significant and perhaps unique regulation of the DC endocytic system at several levels (reviewed in [99]). To begin, pinocytosis and phagocytosis can be curtailed when DCs undergo maturation, through inactivation of a required rhoGTPase [100]. This seems to limit the presentation of peptides to antigens that are captured in the periphery, when the DCs are immature and responding to innate stimuli, and not to self antigens that would be taken up following arrival of the mature DCs in the T cell areas. The lysosomes of certain immature DCs are also unusual relative to other cells, particularly macrophages, in that proteins are degraded slowly by DCs. This reflects two features: a relatively high intravacuolar pH [101] and a relative lack of proteases (rather than other lysosomal acid hydrolases) [102]. When DCs receive a maturation stimulus, a proton pump assembles on the vacuolar membrane, the pH falls to 4.5–5.0, and proteolysis begins (presumably at a limited rate relative to scavenger macrophages). As a result, invariant chain and antigen catabolism are enhanced, thus freeing the MHC class II peptide-binding groove for binding of antigenic peptides. Following the for-

mation of peptide-MHC II complexes within maturing DCs, the complexes move within distinct nonlysosomal vesicles to the cell surface [103, 104]. In the case of bone marrow-derived DCs, these transport organelles also contain the costimulatory molecule CD86, which then remains clustered with the peptide-MHC complexes at the cell surface [103]. Possibly this clustering in maturing DCs translates into efficient and prolonged costimulation of the TCR and CD28 on T cells.

3.5

Uptake of Dying Cells by DCs

The uptake and processing of dying cells provides DCs with the means to present cell-associated antigens to both CD4⁺ and CD8⁺ T cells. When it comes to identifying receptors used by DCs to capture dying cells *in vivo*, there is a striking finding, which is that the CD8 α ⁺ subset of mouse DCs selectively captures autologous dying splenocytes, allogeneic cells killed by NK cells, tumor cells, and virus-infected cells [5, 105]. In contrast, for latex particles, both CD8 α ⁺ and CD8 α ⁻ DCs show comparable phagocytic activity. To date, the mouse is the primary species in which the expression of the CD8 $\alpha\alpha$ homodimer serves as a DC subset marker, but a CD4⁻ subset of rat DCs seems to be an analogous subset that handles dying cells *in vivo* [47].

In macrophages, there are many receptors that can contribute to the uptake of dying cells, but again, much of the evidence has come from *in vitro* approaches. Some receptors that have been implicated in uptake include scavenger receptors [106], the phosphatidyl serine receptor [107], certain integrins [108, 109], CD91/calreticulin [110], CD14 [111], C1qR [112], CD93 or C1qRp [113], and CD36 [114]. For some of these, a role for uptake *in vivo* has been obtained, *i.e.*, for the Mer family of tyrosine kinases [115], the phosphatidyl serine receptor [116], and MFG-E8 [117]. However, the *in vivo* functions that have been studied have not included to antigen presentation.

In DCs, one has a reciprocal situation to the macrophage. There is good evidence for the presentation of dying cells to T cells *in vivo*, but the responsible receptors remain to be identified. Some dying cell receptors in macrophages can be expressed by immature DCs [118–122], but this research has not been extended *in vivo*. As mentioned, a perplexing feature is the selective uptake of certain dying cells by CD8 α ⁺ DCs. CD36, DEC-205, and Langerin are all selectively expressed, but to date, none of these molecules seems essential for the uptake of dying cells [123, 124]. An understudied topic is the death and reprocessing of DCs themselves, which has been documented when DCs arrive in a lymph node and are processed by resident DCs there [4]. The uptake of dying cells *in vivo* is a major area of DC biology and extends to many

clinically relevant topics, since cell death takes place in self tissues, tumors, transplants, and infected cells, allowing DCs to capture antigens for purposes of tolerance and immunity.

3.6

Distinct Endocytic Receptors on DC Subsets

Another feature of uptake receptors is that they can be restricted to subsets of DCs. DEC-205/CD205 and Langerin/CD207 (Fig. 1) are expressed primarily on the CD8 α^+ subset of DCs in spleen and lymph node (although in skin-draining lymph nodes, the LCs that migrate from skin to the node are CD205/207 high but CD8 α low [125]). The CD8 α^+ subset of DCs in mice is also specialized to take up dying cells, and this is postulated to involve distinct uptake receptors. DC-SIGN/CD209 is enigmatic because in mice there is a lack of antibodies to this lectin, but the messenger RNA (mRNA) is primarily found in CD8 α low DCs (H. Hemmi, unpublished) [126]. This area of DC biology is just emerging, but the suggestion is that DC subsets are predetermined to capture distinct ligands through distinct receptors.

3.7

Processing of Glycolipids and Presentation on CD1 Family Molecules

The CD1 family of nonclassical MHC class I-like molecules recognizes various glycolipids, both microbial and self derived. DCs are a major site for the expression of CD1a (Langerhans cells), CD1b and CD1c (dermal DCs, other interstitial DCs, and myeloid DCs) and CD1d (most DCs). The different CD1s seem to capture glycolipids from different endocytic compartments, e.g., CD1a is primarily found in early endosomes, whereas CD1b and CD1c localize to late endosomes [127, 128]. The research on CD1a, b, and c is for the most part limited to ex vivo studies, since these molecules are found in humans, not mice.

Presentation on CD1d is well developed in mice and is increasingly studied in vivo. CD1d presents glycolipids to the invariant TCR on NKT lymphocytes. The glycolipids can derive from endogenous [129], microbial [130, 131], and pharmacologic or synthetic [132–134] sources. An important feature of CD1d presentation by DCs to NKT cells is that it leads to changes in DC function. A single dose of the synthetic glycolipid α -galactosyl ceramide leads to DC maturation and to Th1 CD4 $^+$ and CD8 $^+$ T cell responses to protein antigens that are simultaneously captured [13, 14]. On the other hand, multiple doses of the glycolipid dampen immunity, and this can involve the formation of regulatory IL-10-producing DCs [135]. Since NKT cells can differentiate along

several functionally distinct pathways (Th1 and Th2, T reg), the capacity of DCs to handle CD1 binding glycolipids provides another dimension to the control of immunity.

3.8

Summary

A distinct innate feature of DCs is their endocytic system (Table 2), which helps to explain the efficiency with which these cells translate innate to adaptive immunity. Relatively low doses of antigen often suffice for DCs to present antigens to T cells [69, 70, 92, 136]. DCs express a large number of potential uptake receptors. Some of these already are known to lead to presentation on both MHC class I (the cross-presentation or exogenous pathway) and MHC class II products, and very likely to other presenting molecules like the CD1 family. The endocytic system of DCs is peculiar in its capacity for regulation at many levels during maturation, including the expression of uptake receptors, formation of endocytic vacuoles, and the acidity and therefore activity of the DC vacuolar system. A good deal of cell biology needs to be deciphered. For example, what is the nature of the cross-presentation pathway to MHC class I where DCs seem so adept? Are there specializations for antigen presentation to B cells, since DCs can have direct effects on B cells? What are the consequences of selective expression of specific pattern-recognition receptors on subsets of DCs? How do DCs capture many types of dying cells *in vivo*, and do the dying cells further influence (increase or decrease) DC maturation? Overall, the endocytic system of DCs seems specialized for antigen presentation rather than clearance and scavenging, as is the case of professional phagocytes like macrophages and granulocytes.

4

Costimulatory Molecules of Dendritic Cells

4.1

Definition

The term costimulation embraces many concepts and molecular players. The classical meaning is that costimulation provides a “second signal,” in addition to peptide-MHC or “signal one,” and this second signal leads directly to immunity. The B7 family of molecules is the best studied, but this family also includes negative regulators of T cells, such as PD-L1 or B7-H1. DCs can express high levels of these B7 second signals, but as we will discuss, high expression of B7s by itself may not lead directly to the initiation of immunity.

A further concept is that the absence of costimulation leads to tolerance by anergy or deletion, rather than clonal expansion. DCs are specialized inducers of peripheral tolerance in the steady state, which means that targeting of antigens to DCs greatly increases the efficiency with which tolerance can be induced [69, 70, 92, 137]. Interestingly, tolerance via DCs can require more than the expression of peptide–MHC or “signal one” on DCs. Additional B7 family molecules contribute, such as PD-L1 (a ligand for PD-1, programmed death-1, on T cells) and even CD80/86 (which interacts with the negative regulator CTLA-4 on T cells) [138]. The functional consequences of costimulatory molecules, even if one only considers the B7 family, are therefore intricate and can be both immune enhancing and regulatory.

4.2

The B7 Family

As emphasized by Janeway and Medzhitov, a critical link between innate and adaptive immunity is the presence of pathogen recognition receptors that signal the upregulation of T cell costimulators, and thereby the initiation of adaptive immunity [139]. A lack of two B7 molecules, CD80 and CD86, greatly reduces the immunizing capacity of antigen presenting DCs *in vivo* [140]. However, the situation is proving to be more complicated than the hypothesis that the expression of CD80/86 is sufficient to allow DCs to initiate adaptive immunity.

First, many DCs in lymphoid organs seem to express costimulatory molecules in the steady state, particularly the B7 family as well as CD40. However, the targeting of antigen to these DCs does not lead to a primary immune response, as is evident from studies with dying cells [92] and antigens targeted to the DEC-205 receptor [69, 70]. Therefore, in the steady state, CD86⁺ DCs can be functionally immature, *i.e.*, weak at directing T cell differentiation toward interferon- γ production and cytotoxicity, and in establishing memory. One possibility is that CD86 becomes immunostimulatory on DCs when it is expressed at much higher levels or is physically aggregated with the presented MHC–peptide complexes. DCs express relatively high levels of CD86 and other B7 family molecules relative to other cells [141, 142], and as mentioned, maturing DCs discharge clusters of CD86 with MHC II-peptide complexes, which then persist for many hours on the DC surface [103]. This clustering may allow costimulation to begin.

A second feature is that DCs express “negative costimulators” like PD-L1 or B7-H1. PD-L1 ligates PD-1, which allows DCs in the steady state to mediate peripheral T cell tolerance rather than immunity [138]. Also, DCs upregulate both positive and negative costimulators when they are induced to mature.

Likewise, PD-1 and CTLA-4 typically are upregulated on T cells later in the response to antigen, whereas the positive costimulator CD28 is expressed by naïve T cells.

A third perplexing point relates to the consequence of the upregulation of B7 molecules on DCs during maturation. DCs are striking in the rapidity with which increased expression of B7 family members takes place (4–8 h) and the magnitude of expression, e.g., mature DCs can express ten times the levels of CD86 relative to activated B cells and macrophages [141]. However, new evidence indicates that the heightened expression of CD86 and CD80 is not itself sufficient for the induction of immunity, beginning with the paper by Fujii et al. on DC maturation induced by NKT cells [140]. When DCs took up antigen and simultaneously were induced to mature by NKT cells activated by α -Gal Cer, the DCs induced CD4⁺ and CD8⁺ T cell immunity. Immunization was ablated when the DCs lacked CD40, but it was shown that these CD40-negative DCs nonetheless presented antigen well to CD4⁺ and CD8⁺ T cells and expressed very high levels of CD80 and CD86. Thus, a CD40-dependent maturation event was required beyond signals one (MHC-peptide) and two (high levels of CD80 and CD86), and this was not the production of IL-12 [140]. Likewise, Sporri and Reis e Sousa showed that all DCs in mouse spleen upregulate CD86 in response to *in vivo* administration of TLR ligands, but, in an elegant experiment, they found that only the DCs that responded directly to the TLR ligands—as opposed to bystander DCs—were capable of inducing antibody responses with a Th1 isotype profile [143]. In sum, high levels of B7 costimulators represent a characteristic feature of maturing DCs, but there are additional costimulatory features that control the quality and quantity of the primary immune response and very likely memory.

4.3

The TNF-TNF-Receptor (TNF-R) Family Including CD40

Immature DCs, such as some DC subsets in lymphoid tissues, express the TNF-R family member CD40, but expression is enhanced further with maturation. CD40 ligation mediates many important steps of DC biology, including their development from progenitors [144], migration to and survival within the T cell areas [145, 146], improved presentation on MHC class I molecules [147], production of IL-12 [148], and, as mentioned above, the maturation of DCs to induce combined Th1 CD4⁺ and CD8⁺ immunity [140]. DCs can express many different TNF and TNF-R family members. More research on the functions of these molecules *in vivo* is needed.

4.4

The Notch Family

It is newly recognized that DCs can express members of the notch family including jagged-2 and delta-1. The experiments are, for the time being, in culture, where T cell fate decisions can be determined by the type of notch protein expressed on the DC, e.g., delta-1 for Th1 and jagged-2 for Th2 [149].

4.5

Costimulatory Cytokines

Cytokines are increasingly on the center stage of immunology, including costimulation of adaptive immunity. IL-12 and type I interferons are the best-characterized mediators for such adaptive responses as the development of Th1 type T cells [150], antibodies [151, 152], and cytotoxic T cells [153]. These cytokines also feed back to induce or sustain DC maturation, e.g., IL-12 can recruit NK cells that mature DCs and type I interferons do likewise. IL-6 production by DCs allows effector T cells to overcome suppression by CD4⁺ CD25⁺ T cells [154]. An interesting new subset of DCs is termed “Tip DCs” (for *TNF* and inducible nitric oxide synthase producers) [155, 156]. Tip DCs have the potential to protect against an infection like listeriosis [155], and to cause pathology, as in psoriasis [156]. Therefore, cytokines are likely to be a major contributor to the innate protective functions of DCs and in the translation of innate to adaptive immunity.

4.6

Other Costimulatory Molecules

DCs can express high levels of ICAM-1/CD54 and LFA-3/CD58, which are recognized by LFA-1/CD11a and CD2 on T cells respectively. The reciprocal is also true, i.e., DCs can express CD11a and CD2. DCs express different semaphorins, e.g., semaphorin 4A and 6C, which can increase T cell differentiation toward Th1 [157]. DCs produce chemokines, which not only attract specific subsets of lymphocytes but also may contribute to costimulation [158]. An intriguing finding relates to the T-bet transcription factor, originally discovered as a key to Th1 differentiation in T cells [159]. T-bet is also expressed in maturing DCs, and T-bet knockout DCs are strikingly deficient in inducing Th1-type T cell immunity [160].

4.7

Summary

DCs are powerful accessory or costimulatory cells for T cell responses including the initiation of immunity *in vivo*. DCs can express the highest levels of

the traditional costimulator CD86, as well as a wide range of other accessory molecules, especially TNF family members and cytokines, which influence T cell differentiation and possibly memory. This section has mentioned a few of the enigmas in this field. What is the precise role of heightened CD80 and CD86 expression that occurs during DC maturation? What are the consequences for T cells of individual TNF family members (e.g., OX40L, 4-1BBL, GITR) on DCs? How does the transcription factor T-bet alter DC function? Is the production of inflammatory cytokines (e.g., TNF- α , IL-6, IL-12, interferons) one of the most critical means whereby DCs translate innate to different forms of adaptive immunity? We suspect that the key to the function of DCs in adaptive immunity is not so much the expression of qualitatively distinct costimulators. There are other possibilities, such as quantity and speed of expression, which are in turn coupled to special cell homing and antigen capture/processing functions (above).

5

DC Maturation: The Link Between Innate and Adaptive Immunity

5.1

Definition

The term maturation was first used to describe the development of DCs that is required for the induction of immunity [76, 77]. The initial experiments involved Langerhans cells (LCs), which were found to be weak stimulators of the mixed leukocyte reaction and other T cell proliferative responses to mitogens. The LCs only became strong stimulators after culture in the presence of granulocyte-macrophage colony-stimulating factor (GM-CSF), and this maturation of T cell stimulatory function was accompanied by extensive differentiation, i.e., the appearance and loss of many DC markers and the development of a highly “dendritic” morphology. Strikingly, freshly isolated LCs could capture antigens for presentation to activated T cells, while the mature LCs did not process proteins [80, 81]. These observations, which antedated current ideas about costimulation, clearly distinguished two broad requirements for immunity: an antigen capture step that was carried out by immature LCs and an accessory (later “costimulatory”) function that was carried out by DCs that were surprisingly no longer capable of antigen capture. Shortly thereafter, when monoclonal antibodies to CD86 became available, it was recognized that maturing DCs could upregulate the expression of B7-2/CD86 rapidly and to much higher levels than other cells, e.g., LPS stimulated macrophages and B cells [141, 142]. Many scientists have considered

Table 3 Stimuli that induce features of dendritic cell maturation

Ligands for Toll-like receptors
Other microbial products like cholera toxin, filamentous hemagglutinin
CD40 agonists
Cytokines, e.g., type I interferons, thymic stromal lymphopoietin
Fcγ receptors and relatives including PIRs, TREMs
Necrotic cells, heat shock proteins, urate crystals, high mobility group box protein 1
Innate lymphocytes: NK, NKT, γδT

heightened CD86 expression to be synonymous with maturation. However, as discussed above, an increased level of CD86 is a useful way to monitor DC responses to inflammation or infection, but it is not equivalent to maturation (Sect. 4.2) [140, 143], which occurs in response to TLR ligands and many other stimuli (Table 3).

5.2

Distinct Maturation Stimuli Allow DCs to Initiate Distinct T Cell Responses

A good example of the distinct pathways induced by different maturation stimuli involves the myeloid DCs that are found in human blood. When these cells encounter two distinct stimuli—thymic stromal lymphopoietin (TSLP) vs CD40L—the cells differentiate in a manner that at first glance makes the mature DCs look very similar. These changes involve heightened MHC class II and CD86 expression, and acquisition of a highly dendritic appearance. However, the TSLP DCs cause naïve T cells to differentiate into inflammatory Th2 cells that produce TNF in addition to IL-4, 5, and 13, while CD40L DCs causes naïve T cells to differentiate into Th1 cells [161]. Deeper analysis reveals that the TSLP DCs make distinct chemokines from CD40L DCs (TARC and MDC vs Mig) and fail to make inflammatory cytokines like IL-1, IL-6, and IL-12. While much of the literature on maturation deals with the identification of stimuli that allow DCs to elicit strong Th1 type immunity, it is also necessary to understand how DCs induce a more classical “noninflammatory” Th2 pathway of T cell differentiation, where TNF is not produced. This likely involves a distinct maturation response to some microbial products, like the schistosome egg antigen [162, 163] or certain allergens [164, 165].

5.3

The Consequences of TLR Ligation on DCs

DCs respond rapidly to several ligands for TLRs, which are germline-encoded innate receptors for microbial products. Ligands for TLRs have now been

identified [166, 167], and these are discussed in detail in other chapters of this book. Importantly, there are subsets of DCs that express distinct TLRs. TLR7 and 9, the TLRs that respond to nucleic acids, are primarily expressed on plasmacytoid DCs and mediate the production of large amounts of type I interferons. TLR3 is expressed at highest levels on a subset of myeloid DCs, the CD8 α^+ subset in mouse lymphoid tissues, for example. DC subsets also need to be considered when one generates DCs in large numbers from bone marrow progenitors, as is often done to facilitate research. The use of flt-3L as the hematopoietin, rather than GM-CSF, leads to the development of plasmacytoid DCs as well as cells resembling CD8 α^+ DCs, and these subsets may express distinct TLRs [168, 169].

The prototype readout for a TLR response is the activation of nuclear factor (NF)- κ B and the production of inflammatory cytokines, particularly TNF- α , IL-1, and IL-6. The signal transduction pathways for NF- κ B activation in DCs and other cells originate from either MyD88 and/or TRIF (MyD88-independent) adaptor proteins. More research is needed to understand the value of TNF- α , IL-1, and IL-6 on adaptive immunity, but one possibility is that these account for the ability of mature DCs to overcome suppression [154] yet at the same time promote the expansion of existing suppressor T cells specific for self and environmental antigens [170, 171].

For adaptive immunity to develop, additional transcription factors and cytokines are vital. Two cytokines have significant immune-enhancing effects on T cells. IL-12, whose production is enhanced by the transcription factor IRF-5 [172], acts on CD4 $^+$ T cells to enhance Th1 differentiation. Type I interferons (many α -interferons and a single β -interferon), whose production is enhanced by the transcription factors IRF-3 and IRF-7 [173], act on CD8 $^+$ T cells [153, 174] and B cells [151], to enhance the development of cytotoxic T lymphocytes (CTL), memory, and antibody formation. For several RNA viruses, interferon production is likely to proceed via an intracellular receptor, RIG-I, rather than a TLR [175, 176]. The influence of the type of antigen-presenting cell on the pivotal production of IL-12 and interferons needs to be studied more *in vivo*.

Importantly, the differentiation of helper T cells can be influenced by the type of TLR ligand that acts on DCs. CpG DNA in mice, a TLR9 ligand, can be a strong adjuvant for Th1-type immune responses [177] while the TLR7 ligand imiquimod has similar properties [178]. The TLR2 ligand, Pam3Cys, and the TLR5 ligand, flagellin, in contrast, have been reported to induce Th2-type responses [179–181]. Since TLR ligands act on many different types of antigen-presenting cells, it will be important to dissect the immune responses that are induced when TLR ligands engage each cell type including DC subsets *in vivo*. This area will likely influence vaccine design in the future.

5.4

Negative Regulators of DC Maturation

In addition to positive external stimuli for maturation, DCs are subject to several different pathways of regulation. Negative molecules that can act on both TLR and cytokine receptor signaling are the suppressor of cytokine signaling (SOCS) proteins (reviewed in [182]). Many additional inhibitory pathways for TLR signaling have also been identified (reviewed in [183]). To date these pathways have primarily been studied as regulators of the innate cytokine-producing response rather than adaptive immunity. An important cell surface pathway that suppresses IL-12 production and enhances IL-10 involves CD47 and TSP [184, 185]. Reciprocally, IL-4 can act to dampen IL-10 and increase IL-12 production by DCs [186, 187]. Interestingly, the effects of microbial ligands on DCs may depend on their stage of development, e.g., lipopolysaccharide and bacteria can inhibit the formation and differentiation of DCs from monocytes *in vivo* [188], even though these stimuli are classical inducers of the differentiation of immature DCs.

5.5

Negative Regulation of the Immune Response by DCs

DCs at different stages of maturation are able to expand and/or differentiate different types of suppressive pathways. These include IL-10-producing foxp3⁻ Tr1 cells [189] and CD25⁺ CD4⁺ foxp3⁺ suppressors [170, 171]. A potential suppressive pathway in DCs entails the induction of active indoleamine dioxygenase, which can be toxic to lymphocytes [190–192].

5.6

Ways to Think About the Capacity of DCs to Mediate the Translation of Innate to Adaptive Immunity

DCs are able to produce a number of immune-enhancing cytokines, particularly IL-12 and type I interferons. These molecules likely play critical roles in the link between innate and adaptive T cell immunity [172, 173]. Interferons and IL-12 mediate many pathways for adaptive resistance including antibody responses [151], Th1 CD4⁺ T cell development [193], macrophage activation [194, 195], and cytolytic T cell formation [153]. Another observation with respect to heightened cytokine production is that DCs can express higher levels of required signal transduction proteins, e.g., NF- κ B [196]. In other words, because more NF- κ B is available, TLRs and other transducers of maturation may lead to stronger and more rapid responses in DCs.

While it is possible that DCs, relative to other antigen-presenting cells, express unique membrane-associated costimulatory molecules for the adaptive immune response, it is more likely that DCs simply express higher levels of costimulators like CD86, as well as many different accessory molecules as summarized in Sect. 4. In vivo, the capacity of DCs to localize to the T cell areas and select T cell clones represents a valuable innate property that contributes to adaptive immunity. Another important attribute is the ability of DCs to capture, process, and present antigens, and for long periods. This may provide the time required for T cells to commit to differentiation. A corollary has recently appeared, which is that the stability of an MHC-peptide complex accounts for the immunogenicity of so-called immunodominant peptides [197]. This means that the amount and longevity of MHC-peptide complexes on cells, which is a special property of DCs, will also increase immunity.

5.7

Summary

The thymus produces a diverse repertoire of T cell clones in a resting or naïve state. T cells must then make several critical choices involving peripheral tolerance, development of effector functions, and memory. These decisions are influenced by the maturation of DCs that are presenting antigen. There are many stimuli that induce some features of DC maturation (Table 3). In the case of TSLP and CD40L stimulation mentioned in Sect. 5.2, the two maturation stimuli induce distinct chemokines and cytokines, which helps to explain the different T cell outcomes that the DCs bring about. For many maturation stimuli, particularly for TLR ligands, more research is required to assess their consequences in vivo for the establishment of a protective primary immune response, and for memory. A potentially critical feature of DC maturation is that the sustained presentation of MHC peptide allows time for T cell molecules to be induced and act back on the DCs such as CD40L, OX40, 4-BB, and GITR. At this time, much of the literature involves *ex vivo* studies, TCR transgenic T cells, model antigens, and simple readouts of T cell function. By emphasizing DC biology, one has an opportunity to work directly in vivo and with more demanding antigens. The in vivo control of autoimmune diseases and the establishment of protective immunity and memory represent challenges for future research.

6

Discussion of Some Emerging Links of Innate to Adaptive Immunity via DCs

6.1

Induction of Memory

Memory in the context of infection involves long-lived responsiveness to an antigen, microbe, or vaccine as a result of a primary exposure, but not requiring persistent infection or persistent antigen [198–200]. A new clue comes from experiments in which a protein antigen is targeted via DEC-205 to DCs *in vivo*; the mice exhibit CD4⁺ T cell memory for 6 months [136]. This suggests that it should be valuable to consider the biology of the antigen-presenting cell to understand the establishment of memory, with the targeting of antigens to appropriate DCs being a potentially critical ingredient.

6.2

B Cell Responses

We have concentrated on T cells, but DCs influence B cell responses as well. The traditional pathway via CD4⁺ helper T cells needs to be pursued further. For example, it has recently been demonstrated that the induction of strong CD4⁺ helper T cells by DCs *in vivo* leads to more robust antibody responses to a boost with antigen [201]. This raises the possibility that antibody responses can be induced that will be stronger, longer lived, and of appropriate isotype through better control the DC helper–T cell interaction. Then there are pathways in which DCs interact directly with B cells and may require molecules like BAFF on the DCs [202]. Another example of B cell immunity, which is DC dependent but T cell independent, is stimulation of IgA antibody formation to commensal organisms [203]. In other studies, DCs are able to induce class switching on B cells in the presence [204] or absence [205] of CD40 ligation, the latter through BLys and April TNF family members. This new area of DC–B cell interactions raises some questions. Do DCs have special antigen-presenting capacities for B cells, such as retention and/or display of intact antigens? Do DCs or a subset of DCs have a means to access the B cell area? Do DCs use distinct costimulators to control B cells?

6.3

Mucosal Immunity

We summarized early in this review how DCs are positioned at mucosal surfaces and in mucosal-associated lymphoid tissues to capture antigens. This

is itself remarkable because epithelia are barriers, yet DCs may be readily sampling proteins and particulates. There are other enigmas. One relates to the function of the mucosal draining lymph nodes vs the mucosal-associated lymphoid organs themselves. How do DCs move to the T cell areas in the mucosal lymphoid organ, like the tonsil or Peyer's patch, and does this have different consequences than migration to mucosal draining lymph nodes in the gut, chest, or genital tract? Do DCs contribute to the mechanisms for inducing regulatory T cells at the gut surface, to dampen reactivity to harmless antigens in the intestine? Are there epithelial products that condition the DCs to induce different types of T regulatory cells to suppress environmental reactivity? Reciprocally, how can DC function be switched to induce stronger mucosal immunity, which is a requirement for vaccines against many prevalent infections?

6.4

Peripheral Tolerance

This compendium of review articles emphasizes resistance to infection, but resistance carries a risk that is inherent to the function of maturing DCs. When DCs capture microbial antigens, it is likely that the cells are also presenting many peptides derived from self and harmless environmental sources. As a result, it is important that tolerance mechanisms be in place prior to infection so that the DCs do not induce autoimmunity or chronic inflammation to environmental proteins [206]. It has become apparent that DCs are able to induce different types of peripheral tolerance, and this may appropriately condition the lymphocyte repertoire. Some of these tolerance mechanisms are intrinsic, where DCs induce deletion or anergy [69, 70, 207]. Others may be extrinsic or dominant and involve regulatory or suppressor T cells [170, 171, 189], and T-independent antibodies to IgA in the case of commensal organisms in the intestine [203]. These functions in peripheral tolerance may require the special innate features of DCs that have been reviewed here: the capture and processing of antigens, migration to lymphoid tissues, and we suspect, responses to environmental stimuli that allow DCs to produce IL-10 or transforming growth factor (TGF)- β .

6.5

DC Subsets

Although not emphasized in this review, there are specialized populations or subsets of DCs that exhibit different innate features with respect to location, expression of receptors for antigen uptake, intracellular antigen trafficking

pathways, and responses to environmental signals such as TLR ligands. In peripheral tissues, there are distinct markers on DCs that are associated with epithelial and interstitial sites, as illustrated by different endocytic receptors on epidermal LCs (Langerin/CD207 and DEC-205/CD205) and dermal DCs (DC-SIGN/CD209, mannose receptor/CD206) [208]. In the periphery and in lymphoid organs, there are distinct myeloid and plasmacytoid DCs in many species. Within so-called myeloid DCs there can be clear-cut subsets, the classical one being the $CD8\alpha^+$ and $CD8\alpha^-$ of mouse spleen. What is the *raison d'être* for these subsets? Are DC subsets specialized to respond to distinct classes of microbial insults, e.g., plasmacytoid DCs are designed to respond directly to viral infections with their heightened expression of the TLR7 and TLR9 receptors for microbial RNA and DNA, while some subsets of myeloid DCs are designed to interact with bacterial ligands for TLR 2 and TLR4? Is the $CD8\alpha^+$ subset of DCs designed to interact with dying cells of different types, where dying cells are a major potential source of antigens in self tissues, infectious foci, transplants, and tumors? Do DC subsets cooperate in some instances, e.g., myeloid DCs being better antigen capturing and processing cells and plasmacytoid DCs providing large amounts of adjuvant interferons [209]? Can any DC subset take part in the induction of tolerance, and the differentiation of T cells along Th1 or Th2 pathways, or are certain subsets more dedicated to some of these activities? These questions would all benefit from more *in vivo* approaches to discern what DCs are doing in intact tissues without having to isolate (and thereby perturb) them. This is becoming feasible using an approach in which antigens are delivered selectively to specific uptake receptors expressed by subsets of DCs *in situ* [69, 70, 136, 201, 210].

6.6

Vaccine Biology

Vaccine biology is another underlying theme of this compendium of reviews. The need to discover and develop vaccines against prevalent global infectious diseases emphasizes the need to better understand the link between innate and adaptive immunity. Vaccine biology demands not only that there be a measurable immune response, but also that the immunity be sufficient in quantity and quality to provide protection against a specific pathogen or tumor. Immunology needs to extend its scope from informative but simplified assays in mice to more demanding analysis of protection and memory, including in humans. In HIV/AIDS for example, there still are no reports of reliable immunization of T cells in humans exposed to safe forms of HIV vaccines, let alone trials designed to elicit protective immunity in humans.

The means are now available to exploit DC biology in vaccine discovery and development, something that has not been done in the past. A proposal would be that vaccines need to gain access to DCs that are matured appropriately for the pathogen in question, and that this is most likely to succeed if the vaccine-capturing, mature DCs are located in lymphoid organs, the sites for generating immunity. A new approach to achieve these ends has been put forward. It is to target vaccine antigens to antigen uptake receptors that are expressed by DCs in lymphoid organs along with a maturation stimulus. One method for selective delivery involves using antibodies to DC receptors, in which antibodies are engineered to include vaccine proteins. The first observations with this approach in naïve mice indicate that superior and protective immunity can be achieved by more directly considering DC biology during vaccine design [136, 201, 210].

7

Summary

The innate immune system provides many ways to quickly resist infection (Table 1). The two best-studied defenses in DCs are the production of protective cytokines—like IL-12 and type I interferons—and the activation and expansion of innate lymphocytes. IL-12 and type I interferons influence distinct steps in the adaptive immune response of lymphocytes, including the polarization of Th1-type CD4⁺ T cells, the development of cytolytic T cells and memory, and the antibody response. DCs have many other innate features that do not by themselves provide innate resistance but are critical for the induction of adaptive immunity. We have emphasized three intricate and innate properties of DCs that account for their sentinel and sensor roles in the immune system: (1) special mechanisms for antigen capture and processing (Fig. 1; Table 2), (2) the capacity to migrate to defined sites in lymphoid organs, especially the T cell areas, to initiate immunity, and (3) their rapid differentiation or maturation in response to a variety of stimuli ranging from TLR ligands to many other nonmicrobial factors such as cytokines, innate lymphocytes, and immune complexes (Table 3). The combination of innate defenses and innate physiological properties allows DCs to serve as a major link between innate and adaptive immunity. DCs and their subsets contribute to many subjects that are ripe for study including memory, B cell responses, mucosal immunity, tolerance, and vaccine design. DC biology should continue to be helpful in understanding pathogenesis and protection in the setting of prevalent clinical problems.

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