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Review

Antigen-Presentation Properties of Plasmacytoid Dendritic Cells

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One of the remaining enigmas of the dendritic cell (DC) network is the potential contribution of plasmacytoid DCs (pDCs) to antigen presentation. Although the antigen-presentation capacity of conventional DCs (cDCs) is clearly defined, pDCs are generally attributed as having little, if any, antigen-presentation function. Instead, pDCs are regarded as immunomodulating cells, capable of directing the immune response through their secretion of large amounts of type I interferons. In this review, we examine the evidence for a potential role of pDC in antigen capture, processing, and presentation to T cells at sites of infection and in the lymph nodes.

Introduction

The identification and characterization of dendritic cells (DCs) provided a solution to two major immunological problems that for a long time baffled immunologists (Steinman, 1991). First, it identified the cells required for initiation of T cell-dependent immune responses, a function no other cell type performs as efficiently as DCs. Second, it described a cell capable of transporting to and presenting in the lymph nodes (LNs) antigens captured in peripheral tissues, thereby providing a cellular connection between the likely points of pathogen entry and the organs in which immune responses against those pathogens are initiated. Subsequent work established that the DC network is actually composed of multiple subtypes that vary in hematological origin, life cycle, and functional properties but that share enough features to include them in a single family (Shortman and Naik, 2007; Villadangos and Schnorrer, 2007). One of the recognized members of this family is the plasmacytoid DC (pDC), which is different enough from the rest of the family to be included in a subgroup of its own, distinct from the other subtypes of "conventional DC (cDC)." pDCs were included in the DC family relatively recently (Cella et al., 1999; Grouard et al., 1997; Siegal et al., 1999), but cells with characteristics of pDCs had been known for several decades earlier as "T-cell associated plasma cells," "plasmacytoid T cells" and "natural interferon-producing cells" (Fitzgerald-Bocarsly et al., 2008). The latter name refers to the only undisputedly unique feature of pDCs: their ability to quickly secrete large amounts of type I interferons (IFN I) in response to viral infections, owing to their constitutive expression of the transcription factor IRF-7 (Fitzgerald-Bocarsly et al., 2008). To respond to pathogens, pDCs do not need to be infected. They can detect the unique structural features of viral nucleic acids, such as unmethylated CpG-rich DNA motifs or double-stranded RNA, by employing Toll-like receptors (TLRs). When TLRs engage these motifs, they initiate a signaling cascade that results in pDC activation.

In addition to secreting IFN I, activated pDCs undergo other important phenotypic changes, notably the acquisition of a dendritic morphology and the upregulation of MHC and T cell costimulatory molecules, which enable pDCs to engage and activate naive T cells (Asselin-Paturel et al., 2001; Bjorck, 2001; Grouard et al., 1997; Kadowaki et al., 2001; Nakano et al., 2001; O'Keeffe et al., 2002). These are also the major changes that activated cDCs go through when they detect pathogens, and these changes epitomize the so-called maturation process (Reis e Sousa, 2006). The observation that pDCs also acquire a mature phenotype capable of naive T cell activation is what justified their inclusion in the DC family. Because pDCs appear round in shape rather than dendritic before activation, resting pDCs have also been named "pre-pDCs," reserving the term pDCs only for their activated counterparts (Shortman and Naik, 2007; Soumelis and Liu, 2006). For simplicity, in this review we will stick to the terms immature (or resting) and mature (or activated) to refer to these two stages of pDC development.

By secreting IFN I, pDCs can activate both the innate (e.g., natural killer cells) and the acquired (e.g., cDCs and B cells) arms of the immune system. In addition, it is commonly accepted that upregulation of MHC and T cell costimulatory molecules enable mature pDCs to play a direct role in antigen presentation and T cell activation. The mechanisms employed by pDCs to detect pathogens, and the effects of their IFN I secretion on the immune response, have been extensively described in excellent reviews (Colonna et al., 2004; Fitzgerald-Bocarsly et al., 2008; Liu, 2005). The focus of this review is to examine the evidence for a role of pDCs in antigen capture, processing, and presentation. Necessarily, a review on this aspect of pDCs must use cDCs as the model of reference. This will allow us to evaluate the relative contribution of pDCs to T cell immunity in the context of a typical immune response, which probably involves the simultaneous participation of several DC types (Villadangos and Schnorrer, 2007).

pDC Migration in the Steady State and in Response to Infection

The life cycle and migratory properties of cDC types vary considerably, and this can have a major influence on the role of each type in antigen capture and presentation (Villadangos and Schnorrer, 2007). We will therefore start by recapitulating the migratory properties of pDCs.

The pattern of pDC development and trafficking is quite different from that of cDCs. The precursors of cDCs leave the bone

marrow and disseminate via the blood to lymphoid organs and peripheral tissues, in which they convert into resident and migratory cDCs, respectively (Shortman and Naik, 2007). Newly generated cDCs exhibit an "immature" phenotype dedicated to antigen sampling and are characterized by low surface expression of MHC class II (MHC II) and T cell costimulatory molecules (Villadangos and Schnorrer, 2007). The resident cDCs will spend their entire lifespan in this immature state unless they receive activation signals, in which case they undergo profound changes that culminate in the acquisition of a "mature" phenotype (Wilson et al., 2003). The migratory cDCs traffic from the tissues to the local LNs via the afferent lymph and become mature upon reaching the LNs (Villadangos and Schnorrer, 2007). This migration and maturation occur constitutively, even in the absence of germs or when the cDCs are incapable of responding to TLR signaling (Wilson et al., 2008), suggesting a role for migratory DCs in the transport of peripheral self-antigens to induce T cell tolerance (Reis e Sousa, 2006; Steinman and Nussenzweig, 2002). Most of the cDCs do not leave the spleen or the LNs (see also Alvarez et al. (2008) in this issue of *Immunity*).

In contrast to the cDCs, the pDCs develop fully in the bone marrow and then enter the bloodstream (Shortman and Naik, 2007) (Figure 1). In the steady state, the pDCs are present in the thymus and all secondary lymphoid organs (Asselin-Paturel et al., 2003; Bendriss-Vermare et al., 2001; Okada et al., 2003; Summers et al., 2001), but they are difficult to detect in most peripheral tissues (De Heer et al., 2004; Wollenberg et al., 2002). This has led to the notion that pDCs enter the spleen and LNs through the blood but not via the lymph (Randolph et al., 2008), a view supported by the apparent lack of pDCs in gut and liver afferent lymph collected from cannulated rats (Yrlid et al., 2006). However, a recent report has described pDCs in afferent lymph of noninflamed skin of sheep and pigs and in a similar proportion relative to cDCs to that observed in the blood or the lymphoid organs (Pascale et al., 2008). It is unclear why the pDCs were detected in the lymph of large mammals but not rats; this may have been because of species-intrinsic differences or different amounts of pathogen exposure in the animals used in each study (Pascale et al., 2008). Another explanation might be that although the pDCs can access multiple tissues, they are retained in some but not others. Indeed, although pDCs are rare in the skin (Wollenberg et al., 2002) and the lungs (De Heer et al., 2004), they are abundant in the intestine (Wendland et al., 2007) and the kidneys (Woltman et al., 2007). Perhaps the pDCs that enter the skin leave shortly afterward via lymph, whereas those entering the gut leave more slowly or not at all; this might explain the contrast in pDC numbers in afferent lymph collected from the skin and the gut. The notion that, in the absence of infection, substantial numbers of pDCs enter peripheral tissues before migrating to LNs will need to be corroborated in more experimental systems. If confirmed, this notion has important implications. It means that pDCs may be able to play a more important role in early detection of pathogens in peripheral tissues than is usually appreciated. It also means that pDCs, like migratory cDCs, may contribute to the transport of self-antigens from peripheral tissues to the LNs in the steady state (De Heer et al., 2004). Whether pDCs play a direct role in induction of peripheral tolerance to those antigens remains uncertain, because they do not appear to undergo maturation

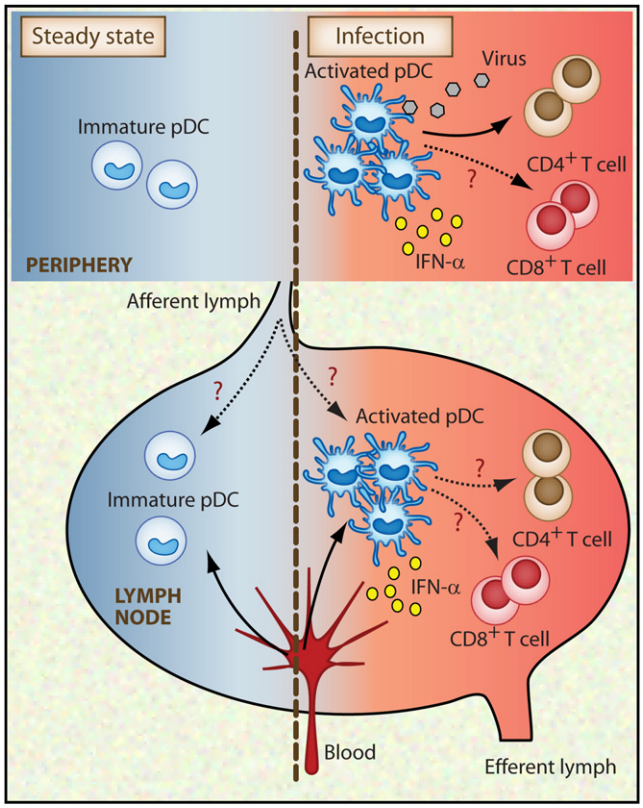

Figure 1. Migratory Behavior and Antigen-Presenting Properties of pDCs in Steady State and Inflammatory Environments
In the steady state, pDCs are produced in the bone marrow and disseminate via the blood to the thymus and the secondary lymphoid organs (spleen, not shown, and the lymph nodes [LNs]). Because pDCs are difficult to detect in most peripheral tissues, it is generally assumed that their primary route of entry into the LNs is through the blood via the high endothelial venules and not by means of the lymph, although new evidence suggests otherwise (see main text for details). Detection of activating signals (e.g., viruses) induces pDCs accumulation at the infection site and associated draining LNs. Activated pDCs secrete large amounts of IFN I and undergo maturation. Mature pDCs acquire dendritic morphology and upregulate MHC and T cell costimulatory molecules, so they have the potential to present antigens via MHC I and II to $CD8^+$ and $CD4^+$ T cells, respectively. However, it is still controversial whether pDCs have a direct role in antigen processing and presentation to T cells, in particular via the MHC I crosspresentation pathway. Such a role appears to be exerted primarily at the inflammation site rather than at the draining lymph node.

(De Heer et al., 2004), a requirement for pDC-mediated T cell stimulation.

Pathogen-associated molecules or inflammatory mediators exert a dramatic effect on pDC trafficking, causing pDC accumulation in the tissues from which these signals are released and in the corresponding draining LNs. For instance, pDCs accumulate (1) in the lungs and mediastinal LNs of mice infected with influenza virus (Geurtsvankessel et al., 2008) or respiratory syncytial virus (Smit et al., 2006; Wang et al., 2006), (2) in the subcutaneous LNs of mice infected in the skin with *Leishmania major* (Baldwin et al., 2004) or in the footpad with herpes simplex virus 1 (Smith et al., 2003), and (3) in the vaginal mucosa of mice infected with herpes simplex virus 2 (Lund et al., 2006; Shen and Iwasaki, 2006). They are also abundant in inflamed human LNs (Cella et al., 1999) and skin lesions (Wollenberg et al., 2002) and are

recruited in large numbers to human or mouse skin treated with the proinflammatory TLR7 ligand imiquimod (Palamara et al., 2004; Urosevic et al., 2005). The simultaneous accumulation of pDCs in infected tissues and draining LNs might be interpreted as further evidence of pDC migration from the tissues to the LN via the lymph. The reality, however, is not so simple. Most of the pDCs accumulating in inflamed LNs enter via high endothelial venules (Cella et al., 1999; Grouard et al., 1997; Yoneyama et al., 2004). Of the pDCs recruited to peripheral infected tissues, few appear to migrate to LNs, and they only do so relatively late after the onset of infection (Geurtsvankessel et al., 2008). Furthermore, whereas viral infection of sheep skin increased the rate of cDC migration from skin to LNs, it did not alter the rate of pDC migration (Pascale et al., 2008). Therefore, the migratory behavior of pDCs is more consistent with a role in antigen presentation and/or immunomodulation at sites of infection or inflammation rather than with a role in antigen transport to the local LNs for presentation to T cells. This latter function appears to be carried out predominantly by the cDCs (Villadangos and Schnorrer, 2007). Simultaneous recruitment of blood pDCs to the LNs draining the infected site probably serves to promote the formation of an immunostimulatory environment via secretion of type I IFN.

T Cell Activation by pDCs

Before reviewing the work that has been performed to address the antigen-presenting capabilities of pDCs, it is important we clarify how we will use some terms. "Antigen presentation" is used in the literature to refer to two related but distinct phenomena. The first refers to the intracellular processes that culminate in exposure of MHC-peptide complexes on the surface of an antigen-presenting cell (APC). For this to happen, the APC has to synthesize, or capture from the extracellular medium, the precursor polypeptide, degrade it to generate antigenic peptides, and load those peptides onto MHC molecules. In its second connotation, antigen presentation refers to events that follow the recognition of MHC-peptide complexes by T cells, for instance T cell proliferation and cytokine secretion. Studies that measure antigen presentation from the latter perspective are less concerned with the intracellular mechanisms underlying formation of MHC-peptide complexes. Such complexes may be artificially generated by incubating the APC with a synthetic peptide or may correspond to allogeneic MHC molecules. We will refer to this second connotation of antigen presentation as T cell activation or stimulation. If the responding T cells are naive, activation requires costimulatory signals in addition to MHC-peptide complexes, and this is usually termed priming to distinguish it from activation of memory T cells or T cell hybridomas, which are considered to be less dependent of costimulation. Antigen recognition by naive T cells does not necessarily lead to an effector immune response. The outcome can also be tolerogenic, leading to differentiation of regulatory or suppressor T cells, T cell anergy, or abortive T cell proliferation, depending on the signals encountered at the time of antigen recognition (Reis e Sousa, 2006; Steinman et al., 2003). Although activation and priming are terms that are usually associated with immunity, in this review we will also use them to refer to the induction of T cell tolerance.

Distinguishing between the two connotations of antigen presentation is important because many studies that reportedly measured "antigen presentation" by pDCs were in fact assessing T cell activation, either in vitro or upon adoptive transfer in vivo. In many of these studies, the MHC-peptide complexes recognized by the T cells were MHC allotypes or were generated by incubation with synthetic peptides. This is not to say that such studies are not important. They have revealed what pDCs might do provided that they present antigens, and this information may be useful to design therapies based on adoptive transfer of peptide-antigen-loaded pDCs. An important limitation of these studies, however, is that they do not necessarily provide much insight on what pDCs actually do in physiological conditions, in which T cell activation must be preceded by antigen processing and presentation.

There has been some controversy regarding whether pDCs are capable of priming. The consensus now is that they can activate naive T cells, as well as memory T cells, if they undergo maturation (Colonna et al., 2004; Liu, 2005). The expression of MHC and T cell costimulatory molecules on activated pDCs is not as high as on their cDC counterparts, and this is probably why pDCs tend to be less efficient at T cell priming than cDCs. Nevertheless, pDCs can stimulate immunity upon adoptive transfer (Salio et al., 2004; Schlecht et al., 2004), or sustain protective responses at sites of infection (McGill et al., 2008), demonstrating their immunogenic potential. Several reports have indicated that pDCs can also induce T cell tolerance, primarily through the induction of regulatory T cells, a capacity they also share with cDCs (Gilliet and Liu, 2002; Goubier et al., 2008 [in this issue]; Ito et al., 2007; Martin et al., 2002; Ochando et al., 2006; Sharma et al., 2007). This may enable pDCs to promote regulatory T cell formation within the infection site to dampen the immune response and prevent immunopathology (De Heer et al., 2004; Smit et al., 2006; Wang et al., 2006). However, as in the case of cDCs, the factors that determine whether the T cells primed by pDCs will differentiate into effector or regulatory T cells remain ill defined.

Because the pDCs are capable of T cell stimulation, it might be expected that to show antigen presentation by this DC type would simply require incubating the pDCs with an antigen and antigen-specific T cells and measuring the subsequent T cell response as a readout of MHC-peptide-complex formation. When this experiment is carried out employing cDCs, detection of MHC-peptide complexes is relatively straightforward. However, pDCs perform very poorly in this type of experiment, even after correcting for the lower T cell stimulatory capacity of pDCs. Indeed, few reports have actually demonstrated an important role for pDCs in antigen presentation either in vitro or in vivo. This speaks of a clear difference that exists in antigen-presentation function between cDCs and pDCs and that cannot be explained only by differences in MHC expression. What is the basis for this difference? In the following sections, we will dissect what we know about the ability of pDCs to deliver different forms of antigen to the MHC I and II presentation pathways and to produce MHC-peptide complexes.

Presentation of Endogenous Antigens by pDCs

There is no question that pDCs present antigens because they express both MHC I and II molecules. What is not clear is how efficient they are at presenting different types of antigen, especially when compared to cDCs. Antigens expressed by APCs

(endogenous antigens) are readily presented if they access the compartments in which proteases generate MHC peptide ligands (Wilson and Villadangos, 2005). These compartments are primarily the cytosol for peptides presented by MHC I molecules and the endosomal route for those presented by MHC II. Virtually any endogenous protein can occur in the cytosol as a full-length protein or a defective ribosomal product (Yewdell and Nicchitta, 2006), so MHC I molecules can potentially present peptides derived from any protein made by the APC, even those that are normally secreted. The repertoire of endogenous proteins that can be presented via MHC II is more restricted because not all these proteins access endocytic compartments. Those that are normally expressed on the plasma membrane, or in the endosomal route itself, comprise the majority of these proteins because they are turned over by proteolytic degradation in endosomes and lysosomes (Wilson and Villadangos, 2005). Cytosolic proteins can also enter endosomes by autophagy (Paludan et al., 2005; Schmid et al., 2007) or other transporting mechanisms (Zhou et al., 2005), and cDCs exploit this route to present cytosolic proteins via MHC II.

Several studies have shown that pDCs efficiently present endogenous antigens via MHC I and II molecules, whether the antigens are constitutively expressed (Krug et al., 2003; Young et al., 2008) or derived from viruses infecting the pDC (Fonteneau et al., 2003; Salio et al., 2004; Schlecht et al., 2004; Young et al., 2008). These studies confirm that the antigen-presentation machinery of pDC is operative and produces peptide-loaded MHC I and II molecules as in cDC and other APCs. Autophagy allows pDCs to detect viral nucleic acids synthesized in the cytosol by employing endosomal TLRs (Lee et al., 2007). It is likely that this mechanism also allows MHC II presentation of cytosolic antigens in pDCs as it does in cDCs (Paludan et al., 2005), but this has yet to be demonstrated.

Mechanisms of Exogenous Antigen Uptake in pDCs

The category of antigens that pDCs seem to present poorly is exogenous—antigens that have to be captured from the extracellular environment. These are the antigens that cDCs present with higher efficiency than any other APCs, with the exception of antigen-specific B cells (see below). There are three features that make cDCs particularly efficient at exogenous antigen presentation: high endocytic activity, ability to retain on their surface long-lived MHC II-peptide complexes, and the capacity to cross-present. How do pDCs compare?

The cDCs can internalize extracellular material by macropinocytosis, phagocytosis, and receptor-mediated endocytosis, the latter two facilitated by the expression of multiple types of receptors (Villadangos and Schnorrer, 2007). This makes cDCs "all-purpose" APCs, capable of capturing virtually any extracellular material (e.g., soluble proteins, glycosylated compounds, immunocomplexes, artificial particles, cells, bacteria, nucleic acids, etc). Overall, pDCs do not appear as endocytic as cDCs, but this is still a matter of contention. Several mouse and human studies concluded that pDCs cannot phagocytose dead cells, zymosan, or artificial particles (Dalgaard et al., 2005; Grouard et al., 1997; Robinson et al., 1999; Stent et al., 2002), but other studies concluded the opposite (Hoeffel et al., 2007; Ochando et al., 2006). This controversy is reminiscent of former discussions on the phagocytic capacity of cDCs; all of these discussions were settled with the realization that phagocytosis is developmentally regulated in this DC type (Steinman and Swanson, 1995). Subtle differences in the material used to measure pDC phagocytosis, or in pDC origin or assay conditions, may account for the contrasting results of different groups. Perhaps the pDCs only phagocytose if they receive signals through specific receptors, and these were not engaged in all studies. Whatever the reason, this remains a matter for future investigation.

Analysis of uptake of soluble proteins by pDCs has provided more consistent results, with several studies concluding that pDCs efficiently capture ovalbumin (OVA) or hen egg lysozyme (HEL) in vitro and in vivo (De Heer et al., 2004; Sapoznikov et al., 2007; Young et al., 2008). Soluble proteins can be internalized by macropinocytosis or receptor-mediated endocytosis. The macropinocytic activity of pDCs, as measured by uptake of dextran or lucifer yellow, is rather poor (Ito et al., 1999; Robinson et al., 1999), so uptake of OVA and HEL is most likely to be mediated by micropinocytosis or a surface receptor.

There has been considerable interest in recent years on the identification of potential antigen receptors expressed by pDCs as well as cDCs because these molecules represent attractive targets for vaccine delivery (Bonifaz et al., 2004; Corbett et al., 2005). Several pDC receptors have been characterized that, when targeted with antibodies coupled to antigens, mediate endocytosis, processing, and presentation of the antigen; examples of such receptors are BDCA-2, Siglec-H, and DCIR (Dzionek et al., 2001; Jaehn et al., 2008; Meyer-Wentrup et al., 2008; Zhang et al., 2006). These molecules are representatives of the C-type lectin (CLR) and sialic-acid-binding immunoglobulin-like lectin (Siglec) families of receptors (Crocker et al., 2007; Robinson et al., 2006), which along with the triggering receptors expressed on myeloid cells (TREM) (Klesney-Tait et al., 2006) may play important roles in pathogen recognition similar to those played by TLRs (Trinchieri and Sher, 2007), NOD-like receptors, and RIG-like helicases (Meylan et al., 2006). However, currently little is known about the natural ligands of most of the CLRs, Siglecs, and TREMs. The ligands may not even be pathogen-associated molecular patterns but self components, and the primary role of these receptors may not be to capture antigens but to trigger immunomodulatory signals. Indeed, BDCA-2, Siglec-H, and DCIR crosslinking initiates a signaling cascade in pDC that inhibits IFN type I production (Blasius et al., 2004; Cao et al., 2007; Dzionek et al., 2001; Meyer-Wentrup et al., 2008; Rock et al., 2007). Thus, the function of these three receptors may be to detect self components released by damaged tissues at sites of inflammation and inhibit IFN I secretion to prevent immunopathology (Swiecki and Colonna, 2007). Similar inhibitory roles have been described for the receptors ILT-7 and NKp44 (Brown et al., 2004; Cao et al., 2006), but again, whether these molecules have an antigen-receptor function remains uncertain.

Another potential pDC antigen receptor is the surface molecule BST-2, also known as CD317 or HM1.24. This is a commonly used marker of pDCs recognized by the mAbs PDCA-1 and 120G8 (Blasius et al., 2006). BST-2 has been recently described as a "tetherin" that holds newly formed virions on the surface of infected cells to limit viral spread (Neil et al., 2008). It also mediates endocytosis of virions, so it may function as a receptor for presentation of antigens contained in viral particles. In support of this hypothesis, an antigen coupled to the mAb PDCA-1 was

presented by pDC (Sapoznikov et al., 2007), but evidence for a function of BST-2 in presentation of natural ligands is lacking.

A group of receptors whose role in antigen capture and presentation is better established are the immunoglobulin receptors (FcRs). FcRII (CD32) is expressed on pDCs, and this receptor has been shown to mediate internalization of immunoglobulins bound to chromatin (Means et al., 2005), Coxsackie virus (Wang et al., 2007), the model antigen KLH (Benitez-Ribas et al., 2006), and the tumor antigen NY-ESO-1 (Schnurr et al., 2005). The DNA immunocomplexes, and the RNA contained in the opsonised virus, could thus access endosomal TLRs (TLR7 and TLR9), trigger pDC activation, and promote IFN I secretion. In the case of chromatin uptake, this mechanism may contribute to the development of systemic lupus erythematosus. It is reasonable to speculate then that the activated pDCs may also present antigens captured with their FcRs, as cDCs do (Boule et al., 2004; den Haan and Bevan, 2002; Kalergis and Ravetch, 2002; Regnault et al., 1999 and reviewed in Nimmerjahn and Ravetch, 2007). Indeed, those studies that did measure MHC II presentation of immunocomplexed antigens (KLH or NY-ESO-1) showed that pDC could present these two antigens (Benitez-Ribas et al., 2006; Schnurr et al., 2005). However, for KLH the efficiency of this presentation was not compared in detail to that of cDCs, whereas NY-ESO-1 was presented an order of magnitude more efficiently by cDCs.

The conclusion of these studies is that, despite the abundance of putative antigen receptors on the pDC surface, which of these receptors, if any, actually contribute to antigen uptake and presentation remains an open question.

Generation and Turnover of MHC II-Peptide Complexes in pDCs

The second specialization that makes cDCs highly efficient APCs concerns the regulation of their MHC II presentation pathway. Immature cDCs constitutively produce and deliver to their plasma membrane MHC II-peptide complexes, but the number of these complexes on the cell surface remains constant because arrival of new complexes is matched by endocytosis and endosomal degradation of pre-existing ones (Cella et al., 1997; Veeraswamy et al., 2003; Villadangos et al., 2001; Wilson et al., 2004; Zwickey et al., 2006 and reviewed in Villadangos et al., 2005). MHC II-peptide complex turnover is regulated by MHC II β chain ubiquitination (Ohmura-Hoshino et al., 2006; Shin et al., 2006; van Niel et al., 2006), a reaction mediated by the ubiquitin ligase March I (De Gassart et al., 2008; Matsuki et al., 2007; Thibodeau et al., 2008; Young et al., 2008), a member of the March family of membrane-bound ubiquitin ligases (also known as c-mir) (Goto et al., 2003; Ohmura-Hoshino et al., 2006). Upon encounter of activation signals, maturing cDCs transiently increase antigen uptake (West et al., 2004) and upregulate synthesis of MHC II molecules, which are preferentially delivered to the endosomal compartments that contain foreign antigens (Blander and Medzhitov, 2006). MHC II synthesis is later downregulated because expression of CIITA, the master transcription factor of the MHC II presentation machinery, is silenced in mature cDCs (Landmann et al., 2001). In step with this change in MHC II synthesis, MHC II-peptide complex turnover is downregulated during cDC maturation (Cella et al., 1997; Veeraswamy et al., 2003; Villadangos et al., 2001; Wilson et al., 2004; Zwickey et al., 2006). This enables mature cDCs to display on their surface long-lived MHC II molecules loaded with peptides derived from antigens captured at the time of activation (Villadangos et al., 2005). However, this comes at a cost: Mature cDCs lose their ability to present via MHC II newly encountered antigens, including those exogenous antigens that they can still endocytose, such as soluble proteins (Young et al., 2007), and endogenous antigens derived from transfected nucleic acids (Gilboa, 2007; Morse et al., 1998) or infecting viruses (Young et al., 2007). It is important to realize that what makes cDCs so efficient at presentation of exogenous antigens is not so much a special capacity to generate MHC II-peptide complexes as is their ability to retain on their cell surface, for long periods of time, those complexes that were generated during the crucial period after antigen uptake and activation, a period during which availability of peptide-receptive MHC II molecules is temporarily boosted through increased synthesis (Villadangos et al., 2005).

The developmental changes in MHC II presentation described for cDCs do not occur in pDCs. First, CIITA transcription is controlled in pDCs by a different promoter (pIII) than the one used by cDCs (pI), and the pIII promoter is not silenced upon pDC activation (LeibundGut-Landmann et al., 2004). MHC II synthesis and peptide loading is thus maintained in activated pDCs (Young et al., 2008). Second, MHC II ubiquitination and turnover are not downregulated in activated pDCs (Young et al., 2008). This means that pDCs lack the ability to accumulate long-lived MHC II-peptide complexes generated shortly after activation. This is probably one, if not the most important, factor that prevents pDCs from presenting limiting amounts of exogenous antigens as efficiently as cDCs. Thus, even in cases in which both pDCs and cDCs capture in the periphery comparable amounts of a "bolus" of inoculated antigen, only cDCs present this antigen in the LN (De Heer et al., 2004). Naturally, if the amount of antigen captured by the pDCs is sufficiently high, presentation is detectable, as probably happens when the antigen is targeted to a surface molecule (Dzionek et al., 2001; Meyer-Wentrup et al., 2008; Sapoznikov et al., 2007; Schnurr et al., 2005; Zhang et al., 2006) or if it is constantly available at a relatively high concentration in the extracellular medium (Young et al., 2008). This may be the reason why pDCs recruited to organ grafts can present alloantigens (Ochando et al., 2006). Importantly, continued MHC II-peptide complex formation and turnover may provide pDCs some advantages over the cDCs. For instance, at sites of infection or inflammation, the pDCs may be able to continually display to newly arriving T cells updated information about the repertoire of exogenous antigens contained in the surrounding environment.

A form of antigen that can be available for long periods and in high supply is that synthesized by the pDCs themselves—endogenous antigens—which, as mentioned above, are efficiently presented by pDCs. The pDCs clearly have an advantage over cDCs when it comes to MHC II presentation of this category of antigens. Whereas cDCs that become infected with viruses after reaching the mature state have compromised their capacity to present endogenous viral antigens via MHC II (Young et al., 2007), infected activated pDCs maintain presentation of these antigens (Young et al., 2008). Again, this may allow pDCs to play a role in MHC II presentation of viral antigens within the infection site rather than in the draining LN (Geurtsvankessel et al., 2008).

In conclusion, there is little doubt that pDCs employ their MHC II antigen-presentation machinery in a manner that is qualitatively distinct to that described for cDCs. This is in itself sufficient evidence to support the notion that the roles of cDCs and pDCs in antigen presentation to $CD4^+$ T cells are not just quantitatively different, but complementary and, most likely, largely nonoverlapping.

Can pDCs Crosspresent?

The third property that makes cDCs highly efficient APCs is their ability to crosspresent, that is, to present exogenous antigens on their MHC I molecules (Wilson and Villadangos, 2005). Not all cDCs appear equally capable of crosspresentation (Villadangos and Schnorrer, 2007). However, the reason for this heterogeneity is controversial. Some studies suggest that crosspresentation requires specialized machinery that is only expressed in some cDC types (predominantly, in mice, the $CD8^+$ DCs [Dudziak et al., 2007; Pooley et al., 2001; Schnorrer et al., 2006]). Other studies suggest that multiple types of cDCs may be capable of crosspresentation provided they capture the antigen employing the right receptor (Burgdorf and Kurts, 2008) or are properly stimulated at the time of antigen capture (Backer et al., 2008; den Haan and Bevan, 2002). Whether pDCs are able to crosspresent is a controversial and unresolved matter. Several studies have shown that mouse pDCs do not possess the capacity to crosspresent (Jaehn et al., 2008; Salio et al., 2004; Sapoznikov et al., 2007) or that their capacity is negligible when compared to cDCs (Shinohara et al., 2006). In at least two of these studies, the lack of crosspresentation could not be attributed to poor antigen uptake because the pDCs presented the antigen via MHC II (Jaehn et al., 2008; Sapoznikov et al., 2007). This conclusion is consistent with a number of reports that showed that crosspriming of $CD8^+$ T cells in vivo against viruses or intracellular bacteria is exerted by cDCs, with no detectable involvement of pDCs (Belz et al., 2005; Belz et al., 2004a; Belz et al., 2004b; Smith et al., 2003 and reviewed in Villadangos and Schnorrer, 2007).

The crosspresenting capacity of human pDCs has only been assessed in vitro. The tumor antigen NY-ESO-1 in soluble form, associated to immunoglobulins or formulated in the adjuvant iscomatrix, was not crosspresented by human pDCs, although at least the immunocomplexed antigen was presented via MHC II (Schnurr et al., 2005). In contrast, two studies have reported crosspresentation of lipopeptides, cell-associated antigens, and viral particles by human pDCs (Di Pucchio et al., 2008; Hoeffel et al., 2007). Strikingly, the crosspresentation pathway employed by the pDCs in each of these studies was different: In the earlier study, the pDCs used the "cytosolic" proteasome-dependent pathway, whereas in the latter, they relied on the "endosomal" pathway (Rock and Shen, 2005). At present it is difficult to give an explanation for the contrasting results of the different mouse and human studies. The same arguments that have been used to explain the differential crosspresentation capabilities of distinct cDC types may be applicable here: Perhaps the pDCs only crosspresent antigens captured via some receptors or if stimulated by yet poorly-defined factors. Whatever such factors might be, the fact of the matter is that currently there is no strong evidence supporting a role for pDC-mediated crosspresentation in vivo.

Conclusion: How "Dendritic" Are pDCs?

The picture that emerges from the studies we have reviewed is sobering: We know that pDCs can have powerful immunomodulatory roles when they display cognate antigens to T cells, and yet we know very little about their real antigen-presenting functions in vivo. Why is this? By definition, pDCs are "professional" APCs, a category of cells that includes not only cDCs but also B cells and macrophages. The antigen-presenting properties of cDCs, B cells, and macrophages, and the role that antigen presentation by these cell types plays on the immune response, are now well characterized. Why is this not the case for pDCs? One reason we would like to suggest is that perhaps the research on pDC function has been too dominated by the view that pDC must play cDC-like roles. However, it is now clear that the way pDCs capture and handle antigens, and exploit their antigen-presentation machinery, is quite distinct from that described for cDCs.

To illustrate the current status of the field, we could imagine how we would think about B cells if we did not know that their primary role as APCs is to present antigens captured with their surface immunoglobulin. Indeed, B cells are as efficient, or more, than cDCs at presenting antigens recognized by this specialized device (Lanzavecchia, 1990), but the antigen-presenting capacity of polyclonal B cells appears extremely poor when compared to that of cDCs (Schnurr et al., 2005). If the B cell receptor were not known, stimulated B cells would appear to be very good producers of a powerful immunomodulatory protein (antibodies), whose production was somewhat connected to the B cell ability to communicate with T cells, but we would not understand the basis for this communication. Perhaps something similar happens with our understanding of pDC function. They may be specialized at presenting a very specific category of antigens, rather than serving a "multipurpose" antigen-presentation function, which is what cDCs do best. We are not suggesting that pDCs play similar roles to B cells (let alone that they should be renamed "B cell-like plasmacytoid dendritic cells"!). However what we would like to propose is that pDCs represent a distinct type of professional APC, different enough from cDCs to consider them a separate entity.

If pDCs are a distinct type of APC, what is their distinct function? One role suggested by the published record might be to present, at the infection site, endogenous antigens derived from viruses infecting the pDCs themselves (Geurtsvankessel et al., 2008; McGill et al., 2008). Another potential role might be to present, again locally, exogenous antigens captured with still uncharacterized receptors, in a manner analogous to B cells. One interesting possibility might be that such antigens are viral particles (virions) (Di Pucchio et al., 2008; Neil et al., 2008), a form of antigen whose presentation has been little studied. Alternatively, the antigens may be self components. In any case, the consequences of such presentation may be to promote T cell immunity or to dampen ongoing immune responses, given that there is evidence supporting either outcome (see above).

Admittedly, these hypotheses are based on a limited number of studies and need further testing to confirm or discard them. Inflammation does not only recruit pDCs, but also monocyte-derived cDCs (Alvarez et al., 2008), and these have also been shown to present viral antigens in the infection site (Wakim et al., 2008). The "acid test" for the suggestion that pDCs are endowed with a unique APC function should be an infection (or

perhaps a tumor model), in which pDCs play a predominant role in T cell activation, or at least a unique complementary role to that played by cDCs or other APCs. Such situations have not yet been described. Indeed, pDC elimination had little impact on $CD4^+$ T cell responses against herpes simplex virus infection of the vaginal mucosa (Lund et al., 2006) and on $CD8^+$ T cell responses against influenza virus infection of the airways (Geurtsvankessel et al., 2008). This may mean that our hypothesis is wrong, or it may mean that we have not yet come across the right model of infection in which pDCs may play a direct role in antigen presentation. After all, the number of models of pathogen infection available to immunologists is rather limited. Studies of additional models may be required if we are to obtain a full understanding of the antigen-presenting functions of pDCs. The questions we have poised in this review will surely be answered in near future; it is likely that some of the answers will be unexpected.

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