

In Vivo Induction of Immune Responses to Pathogens by Conventional Dendritic Cells

María López-Bravo¹ and Carlos Ardavin^{1,*}

¹Departamento de Inmunología y Oncología, Centro Nacional de Biotecnología, CSIC, Campus Universidad Autónoma, Madrid 28049, Spain

*Correspondence: ardavin@cnb.csic.es

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Specific defense mechanisms against pathogens are fulfilled by different subsets of nonmucosal conventional dendritic cells (DCs), including migratory Langerhans cells (LCs), dermal DCs, and resident CD8⁺ and CD8⁻ DCs found in lymphoid organs. Dermal DCs capture antigens in the skin and migrate to lymph nodes, where they can transfer the antigens to CD8⁺ DCs and activate CD4⁺ T cells. Differential antigen-processing machinery grants CD8⁺ DCs a high efficiency in activating CD8⁺ T cells through crosspresentation, whereas CD8⁻ DCs preferentially trigger CD4⁺ T cell responses. Recent findings have revealed the important role played by monocyte-derived DCs (mo-DCs), newly formed during infection, in activating CD4⁺ and CD8⁺ T cells, regulating immunoglobulin production, and killing pathogens. However, a number of controversial issues regarding the function of different DC subsets during viral, bacterial, and parasitic infections remain to be resolved.

Introduction

Over the last decade, mouse experimental models of infection have provided important insights into the identification, origin, and functional specialization of different dendritic cell (DC) subsets (reviewed by Shortman and Naik, 2007). Specific functions related to defense mechanisms against pathogens have been attributed to the main subsets of mouse conventional DCs (cDCs), which include migratory epidermal Langerhans cells (LCs) and dermal DCs, as well as resident CD8⁺ and CD8⁻ DCs found in peripheral lymph nodes (per-LNs) and the spleen (reviewed by Villadangos and Schnorrer, 2007). However, these reports have also led to controversies over the functional specialization of different DC subsets, probably because of the fact that currently available information relies on studies that explored DC-mediated immune responses against a limited number of pathogens and that were performed with widely diverse experimental strategies. In addition, the recent finding that inflammatory monocyte-derived DCs (mo-DCs) are formed de novo during infectious processes at infection sites and lymphoid organs (reviewed by Leon and Ardavin, 2008b) adds an extra level of complexity to the definition of specific functional capacities of DC subsets. Here, we will discuss the current understanding of the functional relevance of nonmucosal cDCs during in vivo immune responses against viruses, bacteria, and parasites.

Migratory and Resident cDCs in Steady State

cDCs involved in defense mechanisms against pathogens comprise all the DC subsets located in antigen-capture areas related to epithelial surfaces (such as the skin and the mucosa of the digestive, respiratory, and reproductive tracts) and secondary lymphoid organs (such as the spleen, LNs, and gut- and bronchial-associated lymphoid tissues). Because the role of mucosal cDCs has been covered in recent reviews on intestinal, pulmonary, and vaginal cDCs (de Heer et al., 2005; Iwasaki, 2007; Johansson and Kelsall, 2005), we will focus on the role of nonmucosal cDCs in the induction of in vivo immune responses against pathogens. Nonmucosal cDCs include those located in

the skin, per-LNs, and spleen, and they can be subdivided into two categories: migratory cDCs and resident cDCs (reviewed by Shortman and Naik, 2007).

Skin migratory cDCs participate in defense mechanisms against pathogens that gain access to the epidermis or the dermis as a consequence of pathogen-specific skin-invasive mechanisms, skin lesions, or inoculation by insects, such as the malaria mosquito or *Leishmania* sandfly. In steady state, migratory cDCs include immature epidermal LCs and immature dermal DCs that remain in the skin until they migrate, in the absence of inflammation or infection, into the per-LNs through the lymph. This migration process involves the upregulation of MHC class II and costimulatory molecules on the surface of LCs and dermal DCs; this implies that in steady state within the per-LNs, these two DC subsets appear in a mature state with regards to their expression of MHC class II and costimulatory molecules (reviewed by Villadangos and Schnorrer, 2007). LCs have been demonstrated to derive from monocytes (Ginhoux et al., 2006). Although indirect evidence also supports a monocytic origin for dermal DCs (reviewed by Leon and Ardavin, 2008b), this hypothesis remains to be conclusively demonstrated. Within the per-LNs of C57BL/6 mice, LCs and dermal DCs represent approximately 35% and 25% of total cDCs, respectively (Kamath et al., 2002). Analyses of BrdU-labeling kinetics of LCs and dermal DCs within the per-LNs of C57BL/6 mice (Kamath et al., 2002) revealed that migratory cDCs have a slow turnover (half-life > 21 days and 12 days for LCs and dermal DCs, respectively).

Studies based on experimental models of cutaneous infection, which use non-genetically modified mice, as well as studies based on models that use genetically engineered mice and that allow LC in vivo tracking or LC conditional ablation (reviewed by Kissenpfennig and Malissen, 2006) concur on the concept that LCs are not directly involved in the induction of pathogen-specific T cell responses (Allan et al., 2003; Filippi et al., 2003; Lemos et al., 2004; Zhao et al., 2003), although a role for LCs in antigen transport to the per-LNs cannot be excluded. Nevertheless, the participation of LCs in immunity against pathogens

under physiological conditions is not fully resolved (Kissenpfnig and Malissen, 2006; Villadangos and Heath, 2005), and here, we will focus on the role of the better understood dermal DCs in immunity against pathogens.

Immature CD8⁺ DCs and CD8⁻ DCs are found in per-LNs and the spleen. CD8⁺ DCs are mainly located within the T cell areas (i.e., the per-LN paracortex and spleen inner white pulp), whereas CD8⁻ DCs are preferentially located in antigen-capture areas (i.e., the per-LN subsinusal layer beneath the subcapsular sinus, as well as the spleen marginal zone). Within the spleen of C57BL/6 mice, CD8⁺ DCs and CD8⁻ DCs represent approximately 25% and 75% of total cDCs, respectively, whereas in the per-LNs, each subset represents approximately 20% of total cDCs (Kamath et al., 2002). Among spleen resident cDCs, CD8⁺ DCs have the fastest turnover (half-life: 1.5 days), indicating that in the steady state, they are replaced within 3 days, whereas CD8⁻ DCs have a lower turnover (half-life: 3 days). In contrast, within the per-LNs, both CD8⁺ DCs and CD8⁻ DCs display a longer lifespan than they do in the spleen, with a half-life of 4.5 days for both subsets (Kamath et al., 2002). CD8⁻ DCs can be further subdivided into CD8⁻CD4⁻ and CD8⁻CD4⁺ DC subsets (reviewed by Villadangos and Schnorrer, 2007), but because no exclusive functions have been specifically ascribed to these subsets regarding their role in immune responses against pathogens, we will not refer to the subsets individually.

On the basis of current knowledge, CD8⁺ and CD8⁻ DCs appear to fulfill their specific defense functions against pathogens essentially within the environment of the per-LNs or spleen and therefore do not appear to migrate through the blood or lymph to other organs. However, DC-activating stimuli, such as Toll-like receptor (TLR) ligands and inflammatory cytokines and chemokines, induce the maturation of resident cDCs, a process that can determine internal migratory processes from antigen-capture areas to the T cell areas within the LNs or the spleen. Interestingly, chemokine-induced migration of DCs from the splenic marginal zone to the inner white pulp was required for bacterial transport to the T cell areas and initiation of CD8⁺ T cell responses against *Listeria monocytogenes* (Aoshi et al., 2008 [in this issue of *Immunity*]). After activation, CD8⁻ DCs migrate from the splenic marginal zone to the inner white pulp, where they interact with antigen-specific T cells (De Smedt et al., 1996). In addition, analysis of the immune response against *Toxoplasma gondii* (Reis e Sousa et al., 1997) suggests that during infection-induced inflammatory reactions, CD8⁺ DCs migrate from the outer to the inner splenic white pulp. This finding contrasts with the concept that in steady state, CD8⁺ DCs are found in the inner white pulp T cell areas. It could therefore be hypothesized that during infection, newly formed CD8⁺ DCs are first located in the outer white pulp, close to the marginal zone, and subsequently migrate to the T cell areas after contact with a pathogen. In this regard, CD8⁺ DCs located in the T cell areas in steady state could be mainly involved in T cell tolerance induction (reviewed by Steinman et al., 2003). Both CD8⁺ DCs and CD8⁻ DCs appear to derive, in the steady state, from a common nonmonocytic-precursor population (Naik et al., 2006), although the differentiation of each subset is differentially regulated by specific signaling pathways (reviewed by Shortman and Naik, 2007). Resident cDCs located in the per-LNs or spleen are involved in immune responses against pathogens that gain access

to these organs through the lymph or blood or against pathogens transported to the per-LNs by skin migratory cDCs. The different migratory and resident DC subsets found in steady state in the skin, per-LNs, and spleen are summarized in Figures 1 and 2. The phenotypic characteristics of these DC subsets are summarized in Table 1.

De Novo Formation of Inflammatory DCs during Infection

Studies performed during acute or chronic infections caused by viruses, bacteria, and parasites have demonstrated a dramatic increase in the number of cDCs present at both the infection sites, such as the skin, and associated lymphoid organs, such as the draining LNs or the spleen (Leon and Ardavin, 2008a; Martin et al., 2002; Serbina et al., 2003; Yoneyama et al., 2001). In these locations, inflammatory cDCs are mo-DCs derived from Ly-6C⁺ monocytes, which are not found in the steady state (reviewed by Leon and Ardavin, 2008b). Monocytes recruited to the dermis differentiate into dermal mo-DCs, which are first located in the dermis but subsequently migrate into the per-LNs through the lymph. Inflammatory Ly-6C⁺ monocytes are recruited to the skin through inflamed dermal venules by a mechanism involving the interaction of P-selectin glycoprotein ligand-1 (PSGL-1) with endothelial P- and E-selectins and the interaction of L-selectin with endothelial peripheral lymph node addressin (PNA_d; Leon and Ardavin, 2008a). Interestingly, inflammatory dermal mo-DCs and dermal DCs found in steady state display similar phenotypic features (Leon et al., 2007), suggesting a monocytic origin for dermal DCs, as discussed above. Ly-6C⁺ monocytes recruited to the per-LNs and spleen during infection-induced inflammation differentiate into LN mo-DCs and spleen mo-DCs, respectively. Ly-6C⁺ monocyte migration to the per-LNs occurs through high endothelial venules by a mechanism essentially involving L-selectin and PNA_d interactions (Leon and Ardavin, 2008a). To our knowledge, no data on the molecules involved in monocyte-endothelial interactions during monocyte migration through the splenic marginal sinus have been reported.

Importantly, although inflammatory DCs are considered to correspond essentially to DCs derived from Ly-6C⁺ monocytes, a strong increase in CD8⁺ and CD8⁻ DC subsets has also been reported during infection (Leon and Ardavin, 2008a; Martin et al., 2002). These observations indicate that inflammatory cDCs also comprise CD8⁺ and CD8⁻ DCs phenotypically similar to those found in steady state, and they suggest that inflammatory CD8⁺ and CD8⁻ DCs are formed de novo in inflammatory foci and lymphoid organs during an infection. Several reports support the hypothesis that CD8⁺ and CD8⁻ DCs formed de novo under inflammation derive from Ly-6C⁺ monocytes (Leon and Ardavin, 2008b; Leon et al., 2007; Leon et al., 2004), although this hypothesis is still a matter of controversy and has yet to be addressed conclusively. The DC subsets found under inflammatory conditions in the skin, per-LNs, and spleen are illustrated in Figures 1 and 2.

Functional Relevance of Pre-existing cDCs versus De Novo-Formed Inflammatory cDCs

Taking into account the fast turnover of per-LN and spleen cDCs (Kamath et al., 2002), the fast increase in cDC numbers observed

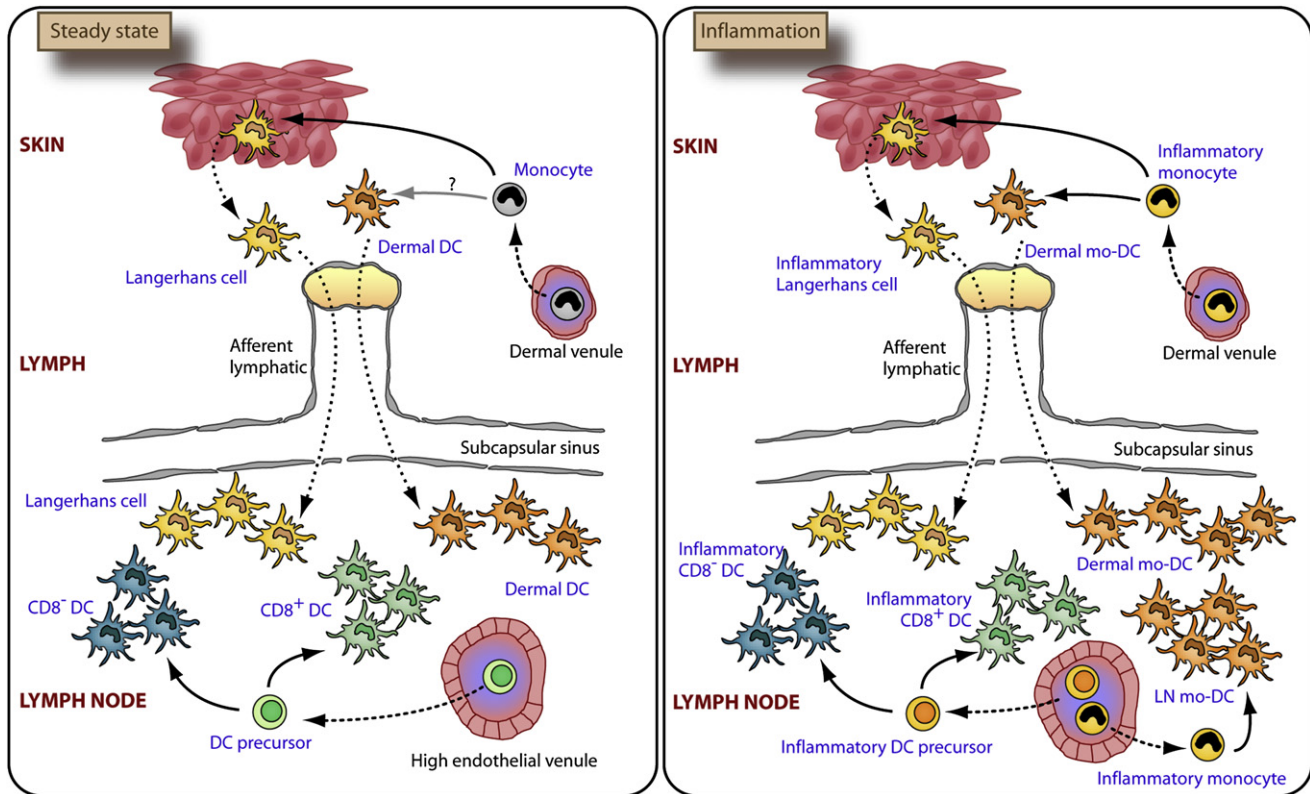


Figure 1. Conventional DC Subsets Present in the Per-LNs in Steady State and during Inflammation

In steady state, per-LN migratory cDCs comprise epidermal LCs differentiated from monocytes and dermal DCs that have also been proposed to derive from monocytes. Both migratory cDC subsets constitutively migrate to the per-LNs and acquire a mature phenotype. Resident cDCs include $CD8^+$ and $CD8^-$ DCs that remain in an immature state in the absence of activating stimuli and differentiate from a common nonmonocytic precursor. During inflammatory reactions caused by infection, pre-existing LCs and dermal DCs rapidly migrate to the per-LNs and are replaced by newly formed LCs and dermal mo-DCs derived from inflammatory $Ly-6C^+$ monocytes recruited to the dermis. Dermal mo-DCs are phenotypically similar to dermal DCs and also migrate to the per-LNs as mature DCs. Whether newly formed LCs migrate to the per-LNs during infection remains to be demonstrated. Inflammatory monocytes are also recruited to the per-LNs and differentiate into mo-DCs. Finally, resident inflammatory $CD8^+$ and $CD8^-$ DCs are also formed during infection, but their precursors have yet to be defined.

after infection (Leon and Ardavin, 2008a; Martin et al., 2002; Serbina et al., 2003; Yoneyama et al., 2001), and the rapid recruitment of monocytes to inflamed foci (Henderson et al., 2003; Le Borgne et al., 2006; Palfaman et al., 2001; Randolph et al., 1999; Serbina and Pamer, 2006), we can conclude that, during infection, migratory and resident cDCs existing before the onset of an infectious process are rapidly replaced by newly formed cDCs. These will include de novo-formed inflammatory mo-DCs, as well as $CD8^+$ and $CD8^-$ DCs. In this regard, studies addressing the role of defined cDC subsets during infection have generally been interpreted on the basis of previous information on migratory and resident cDCs preexisting in steady state. This could have led to misinterpretations due to possible functional differences between cDC subsets in steady state versus inflammatory conditions, such as differences in their migration behavior, antigen-handling capacity, and immunostimulatory potential. Consequently, a number of conflicting points have arisen in regards to de novo DC formation during infection. Are steady-state dermal DCs and inflammatory dermal mo-DCs equivalent? Are $CD8^+$ and $CD8^-$ DCs formed during inflammation functionally equivalent to their counterparts in steady state? Additionally, de novo formation of mo-DCs from monocytes recruited to the per-LNs or spleen has not generally been taken

into consideration when the role of dermal DCs or resident $CD8^-$ DCs during infection has been assessed. Thus, in certain experimental infection models, a specific function that could be fulfilled by dermal mo-DCs or LN mo-DCs could have been ascribed erroneously to dermal DCs. Similarly, some functions that could be carried out by dermal, LN, or spleen mo-DCs could have been attributed incorrectly to $CD8^-$ DCs because in most reports exploring the function of $CD8^-$ DCs during infectious processes, $CD8^-$ DCs were characterized as $CD8^- CD11b^+$, a definition that can also be applied to mo-DCs. These considerations should be taken into account in future studies aiming to ascertain the specific functions of defined DC subsets during infectious processes.

Functional Relevance of $CD8^+$ and $CD8^-$ DCs in T Cell Immunity

Reports published over the last decade point to a functional dichotomy in the role played by $CD8^+$ versus the role played by $CD8^-$ DCs in the induction of immune responses against pathogens. On the basis of these data, $CD8^+$ DCs were claimed to be specialized in the induction of anti-viral $CD8^+$ T cell responses, in part because of their crosspriming potential, whereas $CD8^-$ DCs were proposed to be mainly involved in $CD4^+$ T cell immunity,

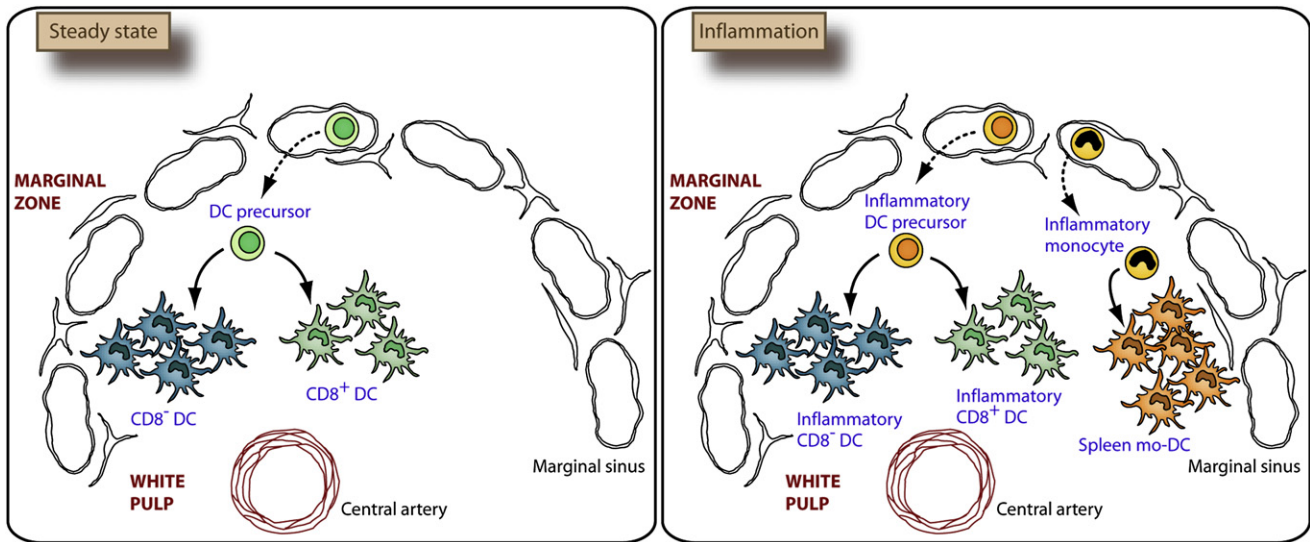


Figure 2. Conventional DC Subsets Present in the Spleen in Steady State and during Inflammation

In steady state, spleen resident cDCs comprise CD8⁺ and CD8⁻ DCs that, as described for their pre-LN counterparts, differentiate from a common nonmonocytic precursor and remain in an immature state in the absence of activating stimuli. During infection, inflammatory monocytes recruited to the splenic marginal zone differentiate locally into spleen mo-DCs, and pre-existing resident cDCs are replaced by de novo-formed inflammatory CD8⁺ and CD8⁻ DCs, derived from an undefined precursor.

particularly during bacterial infections (reviewed by Villadangos and Schnorrer, 2007). This concept has been further supported by a recent report proposing that the preferential ability of CD8⁺ and CD8⁻ DCs to prime CD8⁺ and CD4⁺ T cells, respectively, correlates with the differential capacity of these DC subsets to process and present antigens in association with MHC class I and MHC class II molecules, respectively (Dudziak et al., 2007). In addition, primarily on the basis of in vitro or combined in vivo-in vitro assays, CD8⁺ and CD8⁻ DCs were thought to induce T helper (Th) 1- and Th2-polarized responses, respectively (Maldonado-Lopez et al., 2001; Pulendran et al., 1999).

However, recent studies involving the analysis of in vivo immune responses during infection have challenged these concepts by demonstrating that CD8⁺ DCs are also involved in the induction CD4⁺ and CD8⁺ T cell responses, not only during viral infections, but also during bacterial and parasitic infections (Belz et al., 2005; Maroof and Kaye, 2008; Yarovsky et al., 2006). In addition, under certain experimental conditions, CD8⁻ DCs can produce interleukin-12 (IL-12) (Edwards et al., 2003; Fallarino et al., 2002; Fujii et al., 2003; Maldonado-Lopez et al., 2001) and could therefore be involved in the induction of Th1 cell responses against pathogens.

Table 1. Phenotype of Nonmucosal Conventional DCs

	CD8 ⁺ DCs ^a	CD8 ⁻ DCs ^a	Dermal LCs ^a	Dermal DCs ^a	Dermal mo-DCs ^b	LN mo-DCs ^b
CD11c	+++	+++	++	++	++	++
CD11b	+	+++	+++	++	++	+++
Ly-6C	-	-	ND	++	++	+++
CD8α	+++	-	++	++	++	-
SIRPα	+	+++	++	++	ND	ND
MHC II	++	++	+++	+++	+++	++
DEC-205	+++	-	+++	++	++	-
DCIR2	-	+++	-	-	ND	ND
Langerin	++	-	+++	-/+++ ^c	ND	ND
CD103	++	-	-	++	ND	ND

“-,” “+,” “++,” and “+++” correspond to null, low, intermediate, and high relative expression of the indicated marker, respectively, for the different DC subsets. ND, not determined.

^aPhenotype in steady state for spleen and per-LN CD8⁺ and CD8⁻ DCs and for LCs and dermal DCs after migration into the per-LNs.

^bPhenotype of mo-DCs generated in the dermis and per-LNs.

^cDermal DCs can be subdivided into Langerin⁺ and Langerin⁻ subsets.

Although specific functions related to defense mechanisms against viruses, bacteria, and parasites have been attributed to different subsets of cDCs on the basis of recent advances in DC-mediated immunity against infection, there still are controversial points to be clarified. It is important to bear in mind that current available information on the functional specialization of DC subsets relies on studies that explored DC-mediated immune responses against a limited number of pathogens and that were performed with diverse experimental strategies. In addition, even though some precise functions have been assigned to individual DC subsets on the basis of the expression of C-type lectin antigen receptors (Carter et al., 2006; Dudziak et al., 2007; Sancho et al., 2008), TLR activation receptors (Yarovinsky et al., 2005), and chemokine receptors (Aliberti et al., 2000), a complete definition of specific expression profiles for these receptors and antigen-processing machinery should be performed to accurately delineate the role of each DC subset in defense against infection.

Roles of CD8⁺ DCs and Dermal DCs in Immune Responses against Pathogens

In vivo studies that used mouse experimental models of infection by cytolytic and noncytolytic viruses (Allan et al., 2003; Allan

et al., 2006; Belz et al., 2004a; Belz et al., 2005; Dalod et al., 2002; Mount et al., 2008; Smith et al., 2003; Wilson et al., 2006), bacteria (Belz et al., 2005; Yrlid and Wick, 2002), and parasites (Sponaas et al., 2006; Yarovinsky et al., 2006) strongly support the concept that under physiological conditions, CD8⁺ DCs fulfill a crucial role in the induction of protective Th1 cell responses against intracellular pathogens. Consistent with this concept, CD8⁺ DCs have been shown to be extremely efficient in producing IL-12 in vivo after microbial stimulation (Maldonado-Lopez et al., 2001; Pulendran et al., 1999) and during in vivo infection (Dalod et al., 2002; Maroof and Kaye, 2008; Reis e Sousa et al., 1997; Yrlid and Wick, 2002). Interestingly, CD8⁺ DCs have also been recently demonstrated to induce Th1 cell responses in vivo by an IL-12-independent, CD70-dependent mechanism (Soares et al., 2007).

CD8⁺ DCs can activate CD4⁺ and CD8⁺ T cell responses after internalization of pathogens or pathogen-derived antigens in per-LNs or the spleen, either by direct presentation to CD4⁺ or CD8⁺ T cells or by crosspresentation to CD8⁺ T cells (Allan et al., 2003; Allan et al., 2006; Belz et al., 2004a; Belz et al., 2005; Dalod et al., 2002; Mount et al., 2008; Smith et al., 2003; Sponaas et al., 2006; Wilson et al., 2006; Yarovinsky et al., 2006; Yrlid and Wick, 2002). Interestingly, recent data have led to the concept that during cutaneous infections, skin migrating dermal DCs and per-LN resident CD8⁺ DCs cooperate for the induction of immune responses (Allan et al., 2003; Allan et al., 2006; Belz et al., 2004b). During viral infections, per-LN CD8⁺ DCs have been demonstrated to activate CD8⁺ T cells by crosspresentation of viral antigens transferred to CD8⁺ DCs by dermal DCs that have uptaken those pathogens in the dermis and migrated to the per-LNs; these dermal DCs were responsible for the induction, within the per-LNs, of virus-specific CD4⁺ T cell responses (Allan et al., 2006; Mount et al., 2008). In this regard, several reports support the idea that dermal DCs play an essential role in the induction of CD4⁺ T cell responses against pathogens (Leon et al., 2007; Mount et al., 2008; Shklovskaya et al., 2008). Correspondingly, dermal DCs express different C-type lectin receptors, such as DEC-205, Langerin, and dectin-1, allowing them to internalize exogenous antigens efficiently (Carter et al., 2006; Ginhoux et al., 2007; Shklovskaya et al., 2008). A role for dermal DCs in the crosspriming of CD8⁺ T cells during infection cannot be excluded, and such a role is supported by dermal DCs' expression of the C-type lectin DEC-205 (Shklovskaya et al., 2008) and data on the crosspriming capacity of dermal mo-DCs (Le Borgne et al., 2006).

CD8⁺ DCs are highly efficient in processing and presenting antigens through the MHC class I pathway, either by direct presentation or crosspresentation (den Haan et al., 2000; Dudziak et al., 2007; Schnorrer et al., 2006), and CD8⁺ DCs represent the main DC subset responsible for the induction of CD8⁺ T cell responses against pathogens in vivo, particularly during viral infections (Allan et al., 2003; Allan et al., 2006; Belz et al., 2004a; Belz et al., 2005; Mount et al., 2008; Smith et al., 2003; Wilson et al., 2006). The high crosspriming efficiency of CD8⁺ DCs has been correlated with their ability to internalize dying cell-associated antigens (Iyoda et al., 2002; Schulz and Reis e Sousa, 2002) and with the expression of C-type lectin antigen receptors, such as DEC-205 (Dudziak et al., 2007), CD36 (Tagliani et al., 2008), and the recently described DC, NK lectin group recep-

tor-1 (DNGR-1; Sancho et al., 2008), that target antigens to the crosspriming processing pathway. Consistent with this notion, analysis of gene-expression profiles suggest that CD8⁺ DCs, in contrast to CD8⁻ DCs, are endowed with the antigen-processing machinery required for crosspresentation (Dudziak et al., 2007).

A crucial role for CD8⁺ DCs in the induction of Th1 cell responses has also been demonstrated after infection by the enteroinvasive parasite *Toxoplasma gondii* (Reis e Sousa et al., 1997). Interestingly, IL-12 production by CD8⁺ DCs was triggered after recognition of *Toxoplasma*-derived profilin by TLR11, expressed by CD8⁺ DCs (Yarovinsky et al., 2005). Splenic CD8⁺ DCs efficiently uptake *Listeria monocytogenes* and appear to be essential for bacterial entry into the spleen and subsequent initiation of defense mechanisms against this microorganism (Neuenhahn et al., 2006). Finally, an immunoregulatory function leading to the suppression of T cell responses and the control of excessive inflammatory reactions has been proposed for CD8⁺ DCs; this function depends on a mechanism mediated by the tryptophan metabolism pathway and initiated by the induction of the enzyme indoleamine 2,3-dioxygenase (IDO) (Fallarino et al., 2004). Interestingly, recent data support a role for regulatory T cells in the induction of IDO in CD8⁺ DCs by a reverse signaling mechanism that triggers the noncanonical activation of the transcription factor NF- κ B in CD8⁺ DCs (reviewed by Puccetti and Grohmann, 2007). However, a direct implication of IDO-mediated negative regulation by CD8⁺ DCs during in vivo immune responses against pathogens remains to be demonstrated.

Role of CD8⁻ DCs in Immune Responses against Pathogens

In contrast to CD8⁺ DCs, CD8⁻ DCs are very effective in capturing pathogens and pathogen-derived antigens by phagocytosis and endocytosis and presenting them to CD4⁺ T cells through the MHC class II pathway (reviewed by Villadangos and Schnorrer, 2007). In this regard, CD8⁻ DCs efficiently induce CD4⁺ T cell responses after antigen targeting via C-type lectin receptors, such as dectin-1 and DCIR-2 (Carter et al., 2006; Dudziak et al., 2007). However, CD8⁻ DCs have neither the capacity to internalize apoptotic cells nor to crosspresent antigens to CD8⁺ T cells (den Haan et al., 2000; Iyoda et al., 2002; Schulz and Reis e Sousa, 2002). CD8⁻ DCs express the C-type lectin receptor DCIR2, recognized by the mAb 33D1, that has been proposed to target antigens to the endocytic route involved in antigen presentation to CD4⁺ T cells in the context of MHC class II molecules. This pathway contrasts with the one involving DEC-205 that directs antigens to the processing pathway leading to antigen crosspresentation in the context of MHC class I molecules (Dudziak et al., 2007). Thus, it appears that genes related to the MHC class I processing and crosspresentation machinery were preferentially activated in CD8⁺ DCs, whereas those related to the MHC class II processing machinery were preferentially activated in CD8⁻ DCs. The inefficiency of CD8⁻ DCs at crosspresenting antigens would therefore be due to the lack of the crosspresentation machinery and the lack of expression of C-type lectin receptors, such as DEC-205 or DNGR-1, that target antigens to the crosspresentation pathway.

As mentioned above, data on the induction of polarized Th1 and Th2 cell responses by CD8⁺ and CD8⁻ DCs against soluble

antigens originally led to the notion that CD8⁻ DCs were poor IL-12 producers and were mainly involved in the induction of Th2 cell responses (Maldonado-Lopez et al., 2001; Pulendran et al., 1999). In line with this proposal, in several experimental models of infection by viruses, bacteria, and parasites, CD8⁺ DCs, but not CD8⁻ DCs, were shown to be responsible for IL-12 production and induction of Th1 cell responses (Dalod et al., 2002; Maroof and Kaye, 2008; Reis e Sousa et al., 1997; Yrlid and Wick, 2002). In addition, CD8⁻ DCs have been reported to activate Th2-polarized T cell responses in vivo during infection from *Plasmodium chabaudi* malaria parasites (Sponaas et al., 2006). However, under certain experimental conditions, CD8⁻ DCs can produce IL-12. Thus, IL-12 production by CD8⁻ DCs was demonstrated in response to TLR-7 ligands (Edwards et al., 2003), α -galactosylceramide (Fujii et al., 2003), and LPS in the presence of anti-IL-10 (Fallarino et al., 2002; Maldonado-Lopez et al., 2001). Interestingly, splenic CD8⁻ DCs have been demonstrated to induce CD4⁺ T cell responses during influenza virus infection (Mount et al., 2008) and to induce Th1 cell-biased OVA-specific T cell responses after LPS stimulation by a mechanism that relies on Delta-4 Notch-ligand signaling and is independent of IL-12 production (Skokos and Nussenzweig, 2007).

These data suggest that CD8⁻ DCs could participate in either Th1 or Th2 cell responses depending on different factors, such as the activating stimulus and the internalization, processing, and presentation of pathogen-derived antigens. Unfortunately, only a limited number of reports analyzing the role of CD8⁻ DCs in immunity against pathogens have been published, and consequently, additional experiments should be performed to improve our understanding of the role played by CD8⁻ DCs in immunity.

CD8⁻ DCs have also been proposed to have a microbicidal function through the production TNF- α and iNOS (Serbina et al., 2003) and to participate in the regulation of Ig production by B cells (Balazs et al., 2002). However, on the basis of recent information on inflammatory de novo-formed DCs, it appears that these functions could have been incorrectly attributed to CD8⁻ DCs and could be fulfilled by mo-DCs, as discussed in the next section.

CD8⁻ DCs express the inhibitory receptor SIRP α (Lahoud et al., 2006), which appears to negatively control LC migration (Fukunaga et al., 2004) and has been demonstrated to negatively control IL-12 production after interaction with T cells expressing SIRP α counter-receptor CD47 in human DCs (Latour et al., 2001). This receptor has a crucial role in CD8⁻ DC development because this DC subset is severely reduced in CD47-deficient mice (Hagnerud et al., 2006).

Role of Inflammatory mo-DCs in Immune Responses against Pathogens

mo-DCs differentiated in the skin, per-LNs, and spleen during infectious processes can play an important role in the induction and regulation of immune responses against pathogens (reviewed in Leon and Ardavin, 2008b). Although only a few reports have specifically addressed the role of mo-DCs in innate and adaptive immunity, these studies have demonstrated that mo-DCs can be essential for the induction of effective defense mechanisms against infection. This process applies particularly to nonacute infectious and inflammatory processes that involve the replacement of pre-existing DC subsets by de novo-formed

inflammatory DCs. It is especially important to take into consideration that, as discussed above, some relevant functions that have been assigned to dermal DCs could be actually fulfilled by dermal mo-DCs. These functions could include the capture and transport of antigens to per-LN CD8⁺ DC for crosspresentation, the induction of CD4⁺ T cell responses by direct priming, and, conceivably, CD8⁺ T cell crosspriming. This concept is supported first by the fact that within the per-LNs, both DC subsets have a similar, if not identical, phenotype, and second by the kinetics of dermal DC migration and de novo dermal mo-DC differentiation, which suggests that dermal DCs are rapidly replaced by dermal mo-DCs after the onset of an infectious process.

In line with these considerations, during *Leishmania major* infection, monocytes were recruited to the dermis, draining per-LNs, and spleen and differentiated into dermal mo-DCs, LN mo-DCs, and spleen mo-DCs, respectively (Leon et al., 2007). Dermal mo-DCs acquired a mature phenotype after migration to the per-LNs and were demonstrated to be responsible for the induction of protective CD4⁺ Th1 cell responses against the parasite. Consistent with this finding, during subcutaneous *Salmonella* infection, CD4⁺ T cells were activated by DCs derived from monocytes recruited to the dermis by a CCR6-dependent mechanism (Ravindran et al., 2007). In contrast, LN mo-DCs differentiated from monocytes recruited to the per-LNs during *Leishmania* infection (Leon et al., 2007) exhibited an immature phenotype and did not appear to contribute substantially to T cell immunity against *Leishmania*. The function of LN mo-DCs and spleen mo-DCs during *Leishmania* infection remains to be defined, although it can be speculated that they could participate in innate-immunity defense mechanisms, on the basis of the role of spleen mo-DCs during *Listeria* infection (Serbina et al., 2003). Splenic DCs, formed de novo during infection by *Listeria monocytogenes* from monocytes recruited to the spleen by a CCR2-dependent mechanism, displayed a highly effective microbicidal potential on the basis of their capacity to produce TNF- α and nitric oxide; these mo-DCs have been named Tip DCs (TNF- α - and iNOS-producing DCs). Tip DCs, which displayed a similar phenotype to LN and spleen mo-DCs formed in *Leishmania*-infected mice (Leon et al., 2007), were essential for defense against *Listeria* infection, as demonstrated in CCR2-deficient mice that could not control bacterial replication (Serbina et al., 2003). Interestingly, DCs newly formed in the marginal zone of the spleen during infection by *Streptococcus pneumoniae* were also claimed to correspond phenotypically to Tip DCs (Balazs et al., 2002). These DCs were involved in the induction of T cell-independent B cell responses and in the differentiation of IgM-producing plasma cells by a mechanism relying on the production, by DCs, of the recently described TNF superfamily molecules B lymphocyte stimulator (BlyS) and a proliferation-inducing ligand (APRIL).

These data suggest that whereas mature dermal mo-DCs display an essential role in the induction of pathogen-specific T cell responses, immature LN and spleen mo-DCs would be involved in pathogen killing and participate in the regulation of Ig production by B cells.

Conclusions and Future Research Directions

Recent research on the specific functions fulfilled by different DC subsets during in vivo immune responses have challenged

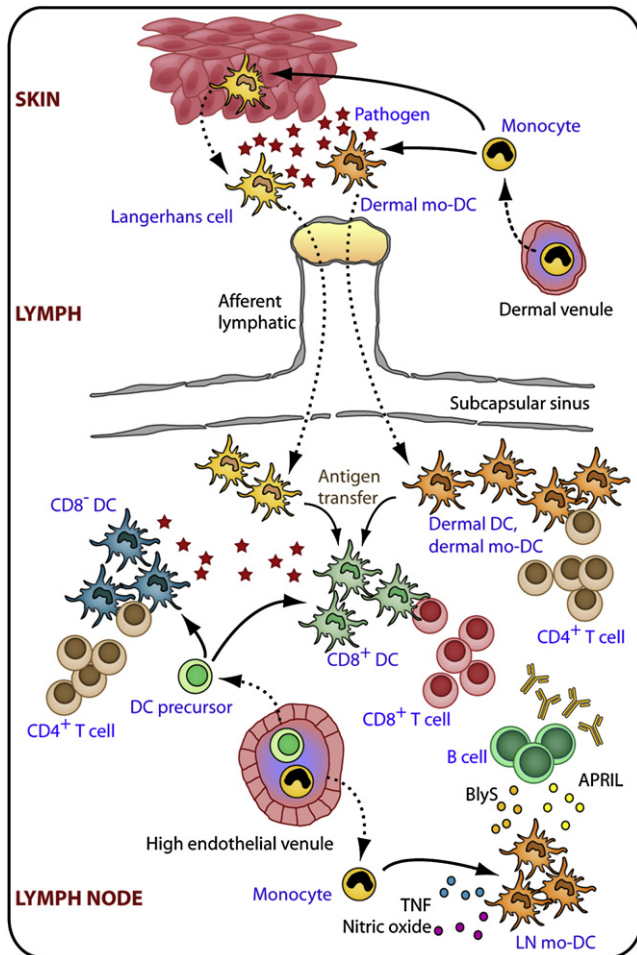


Figure 3. Integrated Model of the Specific Functions Ascribed to the Different Per-LN cDC Subsets during Infectious Processes

Migratory monocyte-derived LCs, dermal DCs, and dermal mo-DCs capture pathogens or pathogen-derived antigens in the skin, transport them to the pre-LNs, and can transfer them to resident CD8⁺ DCs. Both dermal DCs and dermal mo-DCs can also efficiently activate CD4⁺ T cells within the per-LNs. CD8⁺ DCs are highly efficient in activating CD8⁺ T cells by direct presentation or crosspresentation, whereas resident CD8⁻ DCs would preferentially activate CD4⁺ T cells. Finally, LN mo-DCs that display an immature phenotype can have a microbicidal function by producing TNF α and nitric oxide, and they participate in Ig production by B cells through a mechanism dependent on the production of APRIL and BlyS by mo-DCs.

certain paradigms of DC immunobiology, revealed unexpected functions for some DC subsets, and contributed to the description of new DC subsets. Whereas the participation of LCs in immunity against pathogens under physiological conditions represents a yet-unsolved controversy, dermal DCs have recently arisen as critical effectors of immune responses during cutaneous infections by virtue of their capacity to efficiently capture, transport, and present pathogen-derived antigens to per-LN CD4⁺ T cells. Moreover, they enable the crosspriming of CD8⁺ T cells by resident CD8⁺ DCs by providing the latter with pathogen-derived antigens. Importantly, the finding that mo-DCs formed de novo during infection in antigen-capture areas and lymphoid organs can be responsible for the induction of protective immune responses against pathogens has led to the devel-

opment of a new active area of research in DC immunobiology, one that will provide important insights regarding the induction and control of immune responses during inflammation and infection (see Belkaid and Oldenhove, 2008 [in this issue]). An integrated view of the specific functions ascribed to migratory and resident cDCs during infection is shown in Figure 3.

There are numerous controversial and unresolved issues surrounding DC-mediated immune responses against pathogens. Therefore, definitive conclusions cannot yet be drawn on the complete set of functions fulfilled by each cDC subset, functions that appear to be defined by multiple factors, including the control of their differentiation under steady state and inflammation, their location within lymphoid organs, and the expression of specific antigen-capture receptors, TLR and other activating receptors, cytokine and chemokine receptors, and components of the antigen-processing and presentation machinery. Therefore, an in-depth analysis of all these aspects of the immunobiology of the different DC subsets would contribute substantially to the defining of the functional specialization of the different cDC subsets during immune responses against pathogens.

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