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Review

Development and Function of Dendritic Cell Subsets

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Classical dendritic cells (cDCs) form a critical interface between innate and adaptive immunity. As myeloid immune cell sentinels, cDCs are specialized in the sensing of pathogen challenges and cancer. They translate the latter for T cells into peptide form. Moreover, cDCs provide additional critical information on the original antigen context to trigger a diverse spectrum of appropriate protective responses. Here we review recent progress in our understanding of cDC subsets in mice. We will discuss cDC subset ontogeny and transcription factor dependencies, as well as emerging functional specializations within the cDC compartment in lymphoid and nonlymphoid tissues.

Introduction

The vertebrate immune system evolved to react to infection and injury caused by bacteria, fungi, viruses, and immunogenic particles (collectively referred to here as antigens [Ags]) by mounting protective immune responses that improve survival. The highly diverse Ag receptor repertoire required for this broad and almost unlimited reactivity is encoded by T and B lymphocytes. Due to the randomness of the somatic rearrangements generating T and B cell receptors, the repertoire bears reactivity to non-self Ags, as well as self-proteins. Consequently, mechanisms must be in place to avoid fatal autoimmune reactions. Burnet's clonal selection theory proposed that Ag-specific lymphocytes are selected for self- or non-self Ags and undergo clonal expansion upon exposure to the latter (Burnet, 1957). However, early studies based on in vitro "Mishell-Dutton cultures" already postulated accessory adherent, nonlymphoid immune cells required for efficient lymphocyte activation (Hartmann et al., 1970), a notion later substantiated with the discovery of MHC restriction of T cell stimulation (Zinkernagel and Doherty, 1974). Although these accessory cells were first thought to be Metchninkoff's macrophages, studies in the early seventies identified among splenocytes so-called A or 3rd cells that promoted adaptive in vitro immune responses (Cosenza et al., 1971). About the same time, Ralph Steinman discovered in the mouse spleen a rare population of cells characterized by stellate morphology and extended veils (Steinman and Cohn, 1973). He named them dendritic cells (DCs) after the Greek word for tree (*dendron*), but their biological significance met considerable skepticism. It took the persistence of Steinman and his colleagues to provide, over the following years, further compelling evidence for the uniqueness of their novel cell type. In another milestone study they demonstrated that DCs, which prominently express both major histocompatibility complex class (MHC) I and II molecules (Nussenzweig et al., 1980; Steinman et al., 1979), were unrivaled stimulators of T cells in primary mixed leukocytes reactions (MLR) (Steinman and Witmer, 1978). In addition, Steinman showed that DCs could process protein Ag and initiate Ag-specific cellular immune responses (Nussenzweig et al., 1980). For his pioneering work in establishing DC function and biology, as well as his subsequent efforts toward the exploitation of these cells for vaccination, Ralph Steinman was honored in 2011 with the Nobel Prize in Physiology or Medicine.

MHC-II-expressing non-B cells have been identified in almost every tissue investigated, including the intestine, heart, and kidney, with the notable exception of the brain parenchyma. All DCs share the capability to efficiently uptake and process Ags for presentation to naive T cells. However, in the decades since Steinman's seminal discovery, DC subsets have emerged that considerably differ in ontogeny, localization, cytokine secretion pattern, and immunological function. In this review we summarize recent findings and scientific progress in our understanding of murine DC subset development and function. For information regarding human DC subsets, we refer the reader to other excellent recent reviews (Haniffa et al., 2013; Villadangos and Shortman, 2010). We will mainly, but not exclusively, focus on murine classical DC (cDC) subsets, by which we mean all DCs other than plasmacytoid DCs (pDCs) and monocyte-derived DCs (moDCs).

Unifying cDC Features: What Makes These Cells So Special?

Before we discuss individual cDC subsets, we briefly recapitulate some of the unique and potentially unifying features of these highly phagocytic sentinels that seem to have evolved to constantly sense and respond to their immediate environment and communicate with T cells.

Antigen Processing and Presentation

cDCs are specialized in Ag processing and can—probably opposed to most other immune and nonimmune cells—efficiently present endogenous and exogenous Ag in both MHC-I and -II contexts. The unconventional presentation of exogenous noncytosolic Ags on MHC-I by cDCs relies on "cross-presentation" (Bevan, 1976): a phenomenon critical for immunity against viruses and intracellular bacteria (Rock, 2003). The detailed machinery enabling the unique Ags' transport from endosome to cytosol is under intense investigation with different pathways being discussed (Joffre et al., 2012). Unconventional presentation of endogenous cytosolic Ags on MHC-II by cDCs relies in contrast on autophagy (Paludan et al., 2005). Accordingly, this pathway is impaired in ATG5-deficient cDCs, although their Ag cross-presentation remains intact (Lee et al., 2010). As opposed to macrophages, cDCs degrade their engulfed cargo slowly and can control lysosomal degradation potentially to preserve peptides for T cell recognition (Savina et al., 2006). This activity is influenced by the maturation status of the DC, with

lipopolysaccharide (LPS) exposure enhancing lysosomal acidification and Ag proteolysis (Trombetta et al., 2003). Toll-like receptor (TLR) ligand exposure also stimulates Ag macro-pinocytosis, ensuring efficient MHC-peptide (MHCp) formation under inflammatory conditions (West et al., 2004). Interestingly, the glycolytic rate of DCs is also affected by TLR stimulation triggering a circuit that ensures the de novo synthesis of fatty acids critical for proper DC activation (Everts et al., 2014).

Migration

cDCs are strategically positioned at body barriers and also organ entry ports, such as the splenic marginal zone. To ensure stimulation of naive T cells, cDCs require efficient directional migration toward T cell zones either within their respective lymphoid organ of residence or toward remote tissue-draining lymph nodes (LNs). Peripheral cDC migration via afferent lymphatics is CCR7 dependent (Förster et al., 1999) and cDCs utilize CCL19 and CCL21, the same chemokine cues as migrating T cells that enter the LN. Interestingly, mobilization of cDCs can involve, in addition to the chemokine receptor switch, autocrine desensitization by chemokine expression (Dieu et al., 1998). Immobilized CCL21 on, or stored in, lymphatic endothelium plays a critical role in facilitating chemotaxis and arrest of migrating tissue DCs to enter the afferent lymphatics (Tal et al., 2011). Complementary roles in cDC migration have been suggested for other chemokine receptors and S1P1/S1P3 signaling, but CCR7 seems to be the major player. Integrins, on the other hand, are dispensable for the migration of cDCs to LNs under physiological conditions (Lämmermann et al., 2008) but are needed for optimal migration during contact sensitization (Johnson et al., 2006). Intratissue migration of lymphoid organ-resident cDCs, although seemingly also CCR7 dependent, remains less well understood. Recruitment of splenic CD11b$^+$ cDCs to the bridging channels of the marginal zone is controlled by the chemotactic receptor EBI1 (Gatto et al., 2013). A similar scenario was suggested for cDC movement in the Peyer's patch, i.e., the CCR6-CCL20 axis controlling migration toward the mucosal surfaces, whereas the CCR7-CCL19 axis is important for CD8$^+$ DC localization to the T cell region (Iwasaki and Kelsall, 2000). The highly specific expression of the chemokine receptor XCR1 on cross-presenting DCs (Dorner et al., 2009) orchestrates their intratissue positioning in the thymus (Lei et al., 2011). The MHC-II-associated invariant chain (CD74) also seems to be involved in the coordination of DC migration, because CD74-deficient DCs display increased motility, whereas DCs overexpressing CD74 due to absence of cathepsin S migrate at lower speed (Faure-André et al., 2008).

Specialization in T Cell Crosstalk

cDCs have unrivaled potential to stimulate T cells in an MLR in vitro (Steinman and Witmer, 1978). Indeed, studies of mice lacking cDCs, after their constitutive or conditionally ablation, have confirmed the central role of cDCs in the initiation of naive T cell responses (Birnberg et al., 2008; Jung et al., 2002) and the effective restimulation of memory T cells. Importantly though, T cell encounter of MHCp complexes on cDCs has, depending on its context, distinct outcomes. Productive, protective T cell responses, including proliferation, T helper (Th) cell polarization, and memory formation, are believed to rely on three distinct stimuli: cognate MHCp encounter, costimulatory signals provided by B7 family members, and instructing cytokines. All three signals can be derived from DCs for productive T cell priming to occur. Furthermore, these signals seem to have to come from the same DC, because only pathogen-exposed DCs (not inflammation or cytokine-stimulated DCs) can direct full Th cell differentiation (Spörri and Reis e Sousa, 2005). Direct recognition of pathogen-associated Ag by DCs, therefore, seems critical for the initiation of protective T cell responses, suggesting that inflammatory mediators can amplify, but not initiate, adaptive immunity. Such a scenario ensures that T cells read the original context of the cognate Ag, for instance its association with pathogen- or danger-associated molecular patterns. Indeed, it has been proposed that DCs might even maintain the distinction between innocuous Ags and the one received in TLR-ligand context on the single-cell level by segregating their cargo (Blander and Medzhitov, 2006), although this remains to be confirmed in an in vivo setup.

T cell encounter of MHCp on DCs that lack costimulatory molecules contributes to peripheral tolerance (Hawiger et al., 2001; Probst et al., 2003). This notion is supported by the intimate interaction of steady-state T cells and DCs under physiological conditions (Scheinecker et al., 2002). Moreover, DCs can also actively silence T cells by expressing molecules, such as Programmed cell death 1 ligand 1 (PD-L1), which deliver inhibitory signals (Carter et al., 2002). Further evidence for the central role of DCs as critical "hubs" for T cell activation stems from the fact that they are under constant control of thymic and inducible T regulatory (Treg) cells. Relief of this "Treg cell brake" is sufficient to unleash autoreactive cytotoxic T lymphocytes (CTLs) and cause autoimmunity (Feuerer et al., 2009). Interestingly, removal of Treg cells and thus control of DCs also reveals otherwise latent antitumor immunity, as it contributes to the clinical efficacy of costimulation blockades (Marabelle et al., 2013; Vom Berg et al., 2013). Of note, the crosstalk between T cells and DCs is bidirectional; CD40L-expressing T cells critically promote DC "maturation" (Elgueta et al., 2009). Moreover, T cells, as well as innate immune cells, can also shape the cDC compartment by production of the DC poietin Fms-related tyrosine kinase 3 ligand (Flt3L) (Saito et al., 2013; Guermonprez et al., 2013).

Classical DCs

cDCs can be divided into at least two main subsets characterized by either CD8α and CD103 or CD11b expression. Both subpopulations can be found in lymphoid tissue, including spleen, LN, and bone marrow (BM), as well as most nonlymphoid tissue.

CD8α^+ and CD103$^+$ cDCs

Heterogeneity within the DC population was first demonstrated by both the Shortman and Steinman groups, including the discovery of a CD8α-expressing DC subset in murine lymphoid organs (Crowley et al., 1989; Vremec et al., 1992). An equivalent population also exists in nonlymphoid tissues, although these cells do not express CD8 but are instead identified by the CD103 integrin marker ($\alpha_E\beta7$) (Bursch et al., 2007; del Rio et al., 2007) (see below). CD8α^+ and CD103$^+$ cDCs are to date the best-characterized cDC subset, both phenotypically and by gene expression signature (Edelson et al., 2010), and they also appear to be conserved through evolution (Crozat et al., 2010). Indeed, transcriptome profiling allowed the alignment of CD8α^+ lymphoid organ and CD103$^+$ nonlymphoid tissue cDCs