Chapter 4
Functional Diversity of Human Dendritic Cells

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Abstract At the crossroad between innate and adaptive immunity are the dendritic Cells (DCs), a "novel cell type." discovered in 1973 by Ralph Steinman. Although not entirely appreciated at first, it is clear that they play a critical role as specialized antigen-presenting cells and essential mediators in shaping immune reactivity and tolerance. Dendritic cells are now recognized as a heterogeneous group of cells in terms of cell-surface markers, anatomic location, and function adapted to protect against an array of pathogens and conditions. Importantly, these subsets are also unique to each species. While significant progress has been made on the identification and function of mouse DC subsets, much less is known about human cells. Here we review the fascinating biology of human skin DCs and describe tolerogenic principles that are critical in maintaining immune homeostasis and for controlling inflammation, as well as mechanisms that are fundamental to confer immunity. We surmise that these principles could be applied to DCs across organs, and could be harnessed for the treatment of various human autoimmune, inflammatory diseases, as well as cancer. Importantly, to leverage the relevance of basic research to the clinical setting, it is first necessary to determine the functional homology between mouse and human DCs. We discuss practical steps towards this aim.

Keywords Dendritic cells (DCs) · Langerhans cells (LCs) · Cytokines · Dermal DCs · $CD8^+$ T cells · Mouse DCs · Human DCs

Introduction

Dendritic cells (DCs) are key antigen presenting cells that control both immunity and tolerance (Banchereau and Steinman 1998). DCs are localized in surface barriers and function as sentinels for the recognition of pathogens in many tissues. DCs specialize in the capture and processing of antigens in vivo, converting proteins to peptides that are presented on major histocompatibility complex (MHC) molecules for recognition by T cells. Stimulation of innate signaling receptors induce DCs to

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migrate from the periphery to secondary lymphoid organs. This distinguishes DCs from macrophages, which are relatively sessile and largely remain in tissues after inflammation is initiated. Emerging data indicate that peripheral DCs are divided into distinct subsets, each with unique immunological functions. For example, certain DC subsets promote humoral immunity, while others augment cellular immunity. An important contemporary goal is to identify all of the DC subsets and determine their specific functions in immune regulation in humans. The diverse range of evolving pathogens and environmental stimuli resulted in substantial evolutionary changes in the dendritic cell family, as well as within species. Indeed, differences in the basic biology of DCs are only relevant if they prevent us from translating biomedical research to the clinic. It is therefore important to identify functional DC homologs between mouse and human and develop improved preclinical models. Ultimately, targeting or activating distinct DC subsets, with different specializations, will allow controlling the outcome of the immune response. This will be useful for the design of novel vaccines and immunotherapies (Klechevsky and Banchereau 2013).

The Dendritic Cell Discovery and their Exploration in Man

Currently, the central role of dendritic cells in sensing pathogen infection, priming T cell responses and shaping adaptive immune responses is indisputable. But in 1973, when Ralph Steinman first described this "novel cell type" (Steinman and Cohn 1973), the significance of these findings was questioned. Ziegler and Unanue established that the macrophage performed an essential intracellular Ag processing step in T cell activation (Ziegler and Unanue 1981), and with Allen showing that an antigen peptide was presented in the context of MHC molecule in order to activate T cells (Babbitt et al. 1985). So it was established that myeloid cells function as accessory cells to present antigen and produce instructive signals to T cells leading to immune reactivity or tolerance. Two camps formed debating the significance of the dendritic cell in this process relative to macrophages. While both cell types present antigen in the context of MHC, ultimately the dendritic cells were proven to be the most powerful among the APC family in priming T lymphocyte responses.

Langerhans Cells (LCs), dendritic cells of the epidermis, the upper layer of the skin, were first identified in 1868 by Paul Langerhans and were mistaken for nerve cells (Langerhans 1868). The introduction of LCs into the dendritic cell family (Schuler and Steinman 1985) occurred over 100 years later when Schuler and Steinman demonstrated that LCs express MHC-Class II and can induce the mixed lymphocyte reaction. Importantly, this established the concept of "subsets," i.e that DCs, although sharing basic functions, populate different tissues and appear in different forms. The diversity of the DC family was later also explored in the human dermis, where at least three cell populations have been identified (Artyomov et al. 2015; Haniffa et al. 2012; Klechevsky et al. 2008; Nestle et al. 1998, 1993; Zaba et al. 2007).

Peripheral blood is easily accessible from the human body, and because of limited access to human tissue, much of the early work in humans has focused on

blood DCs. However, given the critical function of DCs in barriers, it is important to dissect the instructive signals that DCs produce to maintain tolerance or induce immunity against a challenge in their unique environment. The painstaking task of purifying DC from tissue has made the study of these cells challenging, thus the biological raison d'être for separate mDC subsets in skin has remained largely unknown. Methods for the in vitro generation of large amounts of human DCs from bone marrow progenitors were critical to advance knowledge of the different DC subsets. The in vitro culture of $CD34^+$ hematopoietic progenitor cells with GM-CSF and a variety of cytokines including TNF-a and FLT3-L generated subpopulations of skin-like DCs that expressed HLA-DR and CD1a or CD14. The former expressed features of LCs including Langerin/CD207, E-cadherin and Birbeck granules. Earlier studies (Caux et al. 1997; Morelli et al. 2005) revealed that human skin DCs have not only different phenotype, but also different immunological functions.

Specific Skin DC Subsets Induce Different Types of Immune Responses

DCs are a complex system of cells with different subsets displaying common, as well as distinct functions. The healthy human skin contains multiple myeloid dendritic cell (mDC) populations. Langerhans cells (LCs) are the major DC in the epidermis and DCs in the dermis are identified based on the expression of CD1a and CD14 (Klechevsky et al. 2008; Lenz et al. 1993; Nestle et al. 1993; Zaba et al. 2007). Our studies revealed that different DCs subsets are each endowed with some unique functions. In particular, the $CD14^+$ interstitial DC subset but not LCs promotes humoral immunity by priming of naïve B cells. It was already apparent at that time (Caux et al. 1996) that the $CD1a^+$ -expressing DCs are more powerful at activating T cells compared to the $CD14^+$ DCs. It took 10 years to officially demonstrate that the preferential differentiation of naïve $CD4^+$ T cells in a specific pathway and successful immunity requires not just a sufficient antigenic stimulant, but is also dependent on the DC subsets that is priming the response (Klechevsky et al. 2008). Experimental depletion of a specific DC subset during infection, or during tumor development can impair the immune response. Such facts emphasize that successful immunity largely depends on the DC for appropriate selection of a specific effector module.

We found that the $CD14^+$ DCs prime the activation of $CD4^+$ T follicular helper cells to induce isotype switching and plasma cell generation. LCs preferentially regulate cellular immunity. They do this by inducing Th2 differentiation of $CD4^+$ T cells and by priming and cross-priming naïve $CD8^+$ T cells (Klechevsky et al. 2008). More recently as we extended our studies, we found, together with others, that human LCs are the main skin DC subset to direct IL-17 and IL-22 specific inflammatory responses (de Jong et al. 2010; Fujita et al. 2009; Penel-Sotirakis et al. 2012). It remains unclear how these accessory cells select the appropriate instructive signal for each pathogen. This issue is critical in designing vaccines capable

of driving cell-mediated immunity protective against intracellular pathogens (e.g., HIV, Mtb, malaria) or cancers when Type 1 immunity and CTL responses are desired. We focus on identifying cytokines and transcriptional circuits presented by a unique DC subset that is dominant in instructive T cell differentiation.

Cytokines and Co-Stimulatory Molecules Drive the Functional Specializations of DC Subsets

An essential step in the initiation of an immune response is the formation of the immunological synapse between different antigen presenting cells and T cells via secreted molecules and cell surface receptors. Along with presenting an antigen in the context of MHC Class I and Class II, DCs also provide additional signals to T cells by expressing an array of co-stimulatory molecules and cytokine expression. All these components will eventually determine the outcome of a naïve T cell polarization. Several of the cytokines produced by $CD14^+$ DCs explain their unique capacity to directly signal B cells. These include IL-6, IL-12, IL-10 and TGF-β (Caux et al. 1997, 1996; Klechevsky et al. 2008). IL-12 secreted by DCs upon CD40 engagement is fundamental in initiating IgM production by naïve B cells and IL-12 synergizes with IL-6 and IL-6Rα chain for this effect. IL-10 induces human B cells to switch isotype and to differentiate into plasma cells. The combination of IL-10 and TGF-β stimulates isotype switching toward IgA (Dullaers et al. 2009). IL-6, IL-10 and TGF-β all promote B cell responses in both humans and mice. But IL-12 is an example of a cytokine that has distinct functions in human. IL-12 in human induces IL-21-secreting $CD4^+$ T cells that can induce isotype switching (Schmitt et al. 2013; Schmitt et al. 2009), but has no role in humoral immunity in mice.

LCs Promote Cellular Immunity over Humoral Immunity In our recent work we found that LCs produce IL-15 which enhances the activation of CTLs (Banchereau et al. 2012a). This is important because the combined effects of IL-15 and IL-6 can initiate Th17 responses (Mathers et al. 2009). The role of IL-15 in LC ability to support memory T cells in the skin or activating NK cell is yet to be established.

Dermal $CD14^+$ DCs Promote Humoral Responses by Inhibiting Cellular Responses In our published work, as well as work from another group, it was found that $CD14^+$ dermal DCs not only promote humoral immunity but they actively inhibit the cellular immune response. First, we found that human dermal $CD14^+$ DCs produce IL-10 (Klechevsky et al. 2008), which together with vitamin D promotes $CD4^+$ Treg development (Chu et al. 2012). The secretion of IL-10 and TGF-β impairs the ability of dermal DCs to prime CTLs (Banchereau et al. 2012a). Furthermore, the expression of the inhibitory receptor, specifically, immunoglobulin-like transcript (ILT) receptors ILT2 and ILT4 functions to attenuate cellular immune responses (Banchereau et al. 2012b). ILTs bind MHC Class I and sterically interfere with CD8-binding. This impairs the interaction of the TCR with peptide-MHC complex, block the priming of CTLs, and instead, promotes the generation of Type 2—cytokine secreting $CD8^+$ T cells (Banchereau et al. 2012b).

Translating Dendritic Cell Biology from Mice to Men

While significant progress has been made in the last 5 years on the identification and function of mouse DC subsets, there is relatively little known about human DC subsets. The difficulty in translating mouse studies to human rises from the fact that phenotypically, DC subsets appear to be distinct in each species. This is most likely due to the incredible array of environmental stimuli we are exposed to. Thus, human DC subsets are likely to be more diverse as compared to mouse. Therefore, unlike the study of T cell subsets, attempts to use specific markers to correlate DC subsets between mouse and human have failed. Classification of DC homology based on function is likely to be the only realistic approach for comparing DC subsets across species. This is especially relevant if studies of DC subsets are to lead to targeted therapy.

The Cross-Presenting DC Subset Enigma

Cross-presentation is a process by which antigens are internalized, processed and presented on MHC class I molecules to $CD8^+$ T cells. In mice, this is a critical function of specific DCs in protecting against certain conditions. In human cancer, there is now clinical evidence suggesting that the induction or activation of $CD8^+$ T cells can contribute to the arrest of tumor growth and increase patient survival. In principle, targeting tumor antigens to DCs may enhance protective $CD8^+$ T cell responses due to the ability of DCs to cross-present exogenous antigens (Segura and Villadangos 2009), resulting in an effective T-cell-based vaccine. Specific DC populations in the mouse, the $CD8\alpha^+$ DCs and a related population found in the mouse dermis expressing CD103 and langerin/CD207 are particularly adept in cross-presentation of antigens compared to others (del Rio et al. 2010; Hildner et al. 2008). This finding in the mouse has fueled the quest to identify the counterpart in humans. In 2010, three groups reported the identification of a blood DC subset characterized by the expression of CD141 (BDCA-3/THBD/thrombomodulin). Compared to another DC subset in the blood (the $CD1c^+$ DC) the $CD141^+$ blood DCs are more efficient at engulfing and cross-presenting soluble antigens to $CD8^+$ T cells. This subset shares several markers with the mouse $CD8a^+$ DCs including TLR-3, CLEC9a, and XCR-1 (Bachem et al. 2010; Jongbloed et al. 2010; Poulin et al. 2010; Romani et al. 2010). Interestingly, the capacity to crosspresent is dependent on TLR-activation and the ability of this cell subset to crossprime naïve $CD8^+$ T cells was not evaluated. A recent study indicates that human CD1c (or BDCA-1) expressing cells (that represent the majority of circulating DCs in human blood) can also cross-present to $CD8^+$ T cells as long as the antigen is delivered through a receptor to early endosome (Cohn et al. 2013).

We looked for the counterpart for the mouse $CD8a^+$ DCs in human tissue. Interestingly, blood derived CD141-expressing cells, identified based on their low CD11c expression relative to other dermal DCs, are found infrequently in the human dermis (Haniffa et al. 2012). A similar scenario occurs in mice, where blood derived cells survey the dermis at steady state (Ginhoux et al. 2007). By examining CD141

expression on dermal resident DCs, we noticed that this marker is not restricted to one subset, and nor is the expression of XCR1 or TLR3. Thus the equivalent for the cross-presenting DC subset in the mouse dermis—the CD103+ langerin + DC cannot be identified solely based on these few markers. Furthermore, in humans, Langerin is only expressed on epidermal LCs and CD103 is not expressed on any of the human skin DC subsets, but is instead expressed on T cells. Overall, defining homology between cell types within species based solely on comparing a limited set of markers is likely to be misleading. We favor the idea that functional similarities reflected by gene expression patterns correlated with biological functions will be the best approach for interspecies comparisons of DC subsets.

Interestingly, in our previous work, we examined the genomic profile of the human skin DC by microarray. We noticed that LCs express genes related to MHC Class I antigen processing and presentation machinery, while the dermal $CD14^+$ DCs displayed enhanced expression of the MHC Class II presentation machinery (Banchereau et al. 2009). A similar gene expression profile was also shown for the mouse $CD8a^+$ DCs and the $CD4^+$ DCs, respectively (Dudziak et al. 2007). This suggested that human LCs share similar functional properties with the mouse $CD8a^+$ DCs. Indeed, this was also ultimately proven in a recent study that we performed to identify functional similarities between human skin and the mouse DC systems. The study which included analyses using genomic systems biology alongside functional approaches was published after this chapter was sent to production (hence details are not included) (Artyomov et al. 2015).

Another important consideration, when cmparing the mouse and human systems, is the ability to induce primary responses (the hallmark of dendritic cells) vs. activating secondary responses. Cross-priming occurs when the antigen is presented to a naïve $CD8^+$ T cell resulting in the initiation of a specific immune response, rather than reactivation of an existing memory T cells. In the mouse, thymic but not splenic $CD8\alpha^+$ DCs can cross-prime antigen to naïve $CD8^+$ T cells (Dresch et al. 2011). Functional differences of that kind need to be worked out in the human system.

T Helper Polarization by Mouse and Human DC Subsets

Identifying functional homologs between the mouse and human DCs with respect to the ability to initiate different modules of $CD4^+$ helper responses has only hardly been examined. In the human dermis the $CD14^+$ DCs can activate T regulatory cells through their IL-10 production, a function shared with the mouse dermal CD11b+ DCs and mouse LCs (that also express CD11b). IRF4-dependent DCs (CD11b + CD103-PDL2+ in the mouse and LCs in the human) specialize in regulating Th2 cell responses (Gao et al. 2013; Kumamoto et al. 2013) (Klechevsky et al. 2008) (Fig. 4.1). Both mouse and human LCs can activate Th17 responses. In the mouse, this type of a polarization is dependent on LC-derived IL-6, a cytokine that is not produced by human LCs. Thus, the means by which Th17 cells are being induced by human LCs is yet to be established. Overall, delineation of accurate functional homologies between human and mouse DC subsets requires broad and unbiased systemic comparisons (Crozat et al. 2010; Robbins et al. 2008).

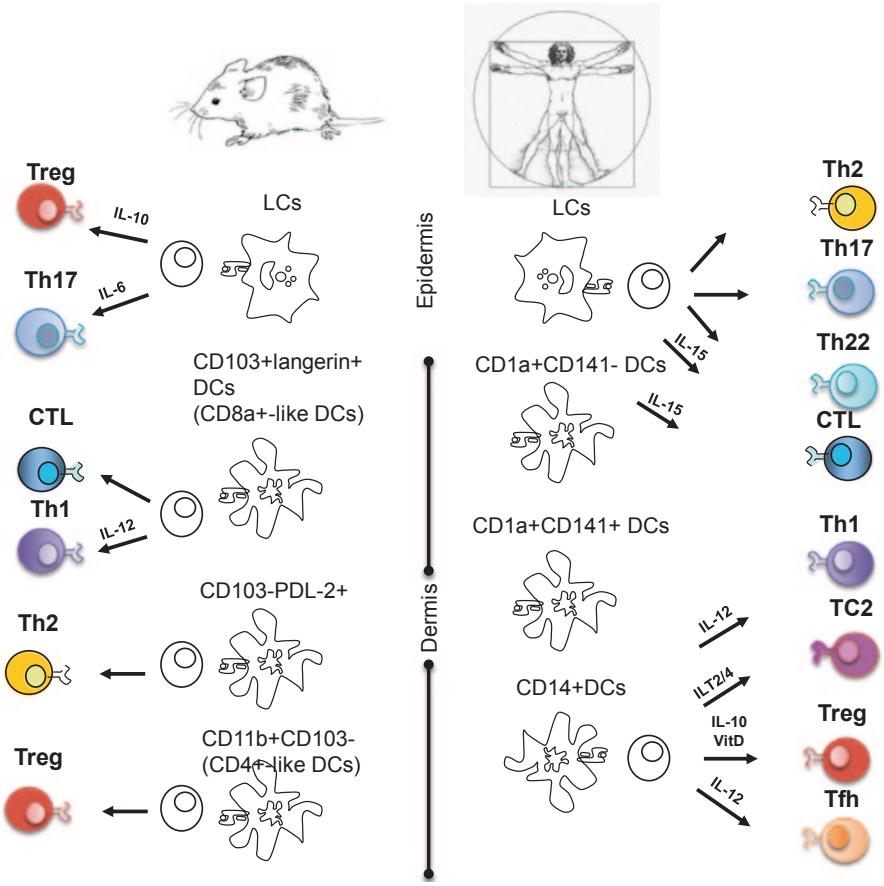

Fig. 4.1 Functional specializations of cutaneous epidermal and dermal dendritic cells in mouse and human

A Controversial Role for Langerhans Cells in Mouse and Human

Human LCs are the most efficient among the dendritic cell subsets in the skin at inducing primary $CD8^+$ T cell responses, by cross-presenting and cross-priming antigens to $CD8^+$ T cells. However, the relevance of LCs to $CD8^+$ T-cell-mediated immunity in mice is still an object of controversy. Particularly since a $CD8a^+$ DC-like population, the $CD103^+$ DCs in the mouse dermis are the most efficient at inducing $CD8^+$ T-cell responses. Although this subset expresses Langerin, it is developmentally distinct from LCs (Nagao et al. 2009). Interestingly, after a 3-day in vitro maturation/differentiation process, murine LCs become very efficient stimulators of T cells suggesting that purified mouse LCs may require an additional signal in comparison to human LCs that can activate T cells immediately following their

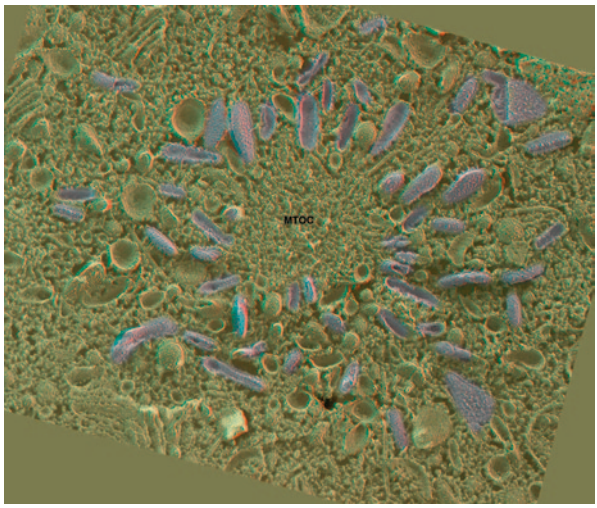

Fig. 4.2 Electron micrograph of Birbeck granules clustering around the cell microtubule-organizing center. (Picture curtsey of John Heuser. Studies were performed in collaboration with Ira Mellman at Yale University)

isolation from tissue (Klechevsky et al. 2008). The identification of two different types of mouse LCs (Sere et al. 2012; Wang et al. 2012), one that is an inflammatory and is present for the short term may be important in the interpretation of studies performed with mouse LCs.

Human LCs do share some similarities with their rodent counterparts. Their physical location as well as the expression of some proteins are similar. For example, Langerin and the associated Birbeck granule (Fig. 4.2), as well as E-cadherin and Epithelial cell adhesion molecule (EpCam) are similarly expressed. However, previously identified differences between mouse and human LCs may help explain the functional discrepancy noted for LCs in the two species. For example, in the mouse, LCs resemble tissue-resident macrophages; they are developmentally dependent on M-CSFR (Ginhoux et al. 2006; Wang et al. 2012), express macrophage-specific markers such as F4/80 and CD11b, migrate poorly to lymph nodes relative to the migration of conventional DCs to lymph nodes, constitutively secrete IL-10 and IL-6 upon activation (Igyarto et al. 2009) and have little to no expression of Zbtb46, which suggests a macrophage identity rather than a DC identity (Meredith et al. 2012; Satpathy et al. 2012). In contrast, human LCs do not express any of the macrophage markers, are negative for CD11b and express IL-15, but not IL-6 or IL-10 (Banchereau et al. 2012a; Klechevsky et al. 2008).

Toll-like receptors are important innate immune sensors. Murine LCs express TLR2, TLR4 and TLR9, but not TLR7 or TLR-3. While human LCs express high levels of transcripts for TLR1, TLR3, TLR5, TLR7 and TLR-8 and weak or absent expression of TLR2, TLR4 or TLR9 (Flacher et al. 2006; Klechevsky et al. 2009). These data suggest that there are interspecies differences in the repertoire of TLR receptors expressed by LCs. Overall human and mouse LCs have evolved differently likely because of distinct microbiota and distinct pathogens.

Concluding Remarks

A transformational change in the treatment of a disease is the ultimate way for demonstrating the value of a basic scientific discovery. Studies in mice have taught us that targeting/depleting a specific DC population can influence the outcome of a disease (Hildner et al. 2008; Hochheiser et al. 2013). The distinct phenotypic and functional divergence among DCs across species limits the direct translation of mouse studies to human. Exploiting the functional diversity of DC subset in humans will be the key to our understanding of human disease processes and viable treatment options. Use of human tissue or the study of patients undergoing efficient therapy is critical and may increase the predictive value of animal models leading to better human clinical trial outcomes. "We count on you," were Ralph's recurrent words to me, urging us scientists in the DC field and other areas of immunology to collaborate and to harness knowledge of this novel cell-type into a novel force in medicine. This is a legacy that we are committed to follow.

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