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Targeting Human Dendritic Cell Subsets for Improved Vaccines

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Summary

Dendritic cells (DCs) were discovered in 1973 by Ralph Steinman as a previously undefined cell type in the mouse spleen and are now recognized as a group of related cell populations that induce and regulate adaptive immune responses. Studies of the past decade show that, both in mice and humans, DCs are composed of subsets that differ in their localization, phenotype, and functions. These progresses in our understanding of DC biology provide a new framework for improving human health. In this review, we discuss human DC subsets in the context of their medical applications, with a particular focus on DC targeting.

1. Introduction

Dendritic cells (DCs) are central players in the control of immunity and tolerance. DCs reside in peripheral tissues where they are poised to capture antigens. Antigen (Ag)-loaded DCs migrate from tissues through the afferent lymphatics into the draining lymph nodes, and then they present processed protein and lipid Ags to T cells via both classical (MHC class I and class II) and non-classical (CD1 family) antigen presenting molecules [1]. The soluble antigens also reach the draining lymph nodes through lymphatics and conduits, and are presented by lymph-node resident DCs [2]. Upon activation, antigen-loaded DCs are geared towards the launching of antigen-specific immunity [3], leading to the T cell proliferation and differentiation into helper and effector cells with unique functions and cytokine profiles. DCs are also involved in the generation of antibody responses, partly through direct presentation of antigens to B cells [4–7]. DCs appear to be also essential for both central tolerance in the thymus and peripheral tolerance. DCs can induce immune tolerance partly through T cell deletion and partly through activation of regulatory T cells (Tregs) [8].

DCs are composed of multiple subsets with distinct functions. Both mice and humans have two major types of DC: classical DCs (cDCs) and plasmacytoid DCs (pDCs). cDCs and

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pDCs are further composed of distinct subsets, which add another layer of complexity in the coordination of immune responses. Functionally distinct cDCs subsets were originally found in mouse spleen, where CD8 α^+ DCs induce Type 1 responses, while CD8 α^- DCs induce Type 2 responses [9,10]. A recent study further demonstrated that CD8 α^+ DCs preferentially induce antigen-specific CD8 $^+$ T cell immunity, while CD8 α^- DCs preferentially induce antigen-specific CD4 $^+$ T cell immunity [11]. In this review, we discuss recent progresses in the determination of phenotypic and functional differences of the known human DC subsets. We also discuss how we translate knowledge obtained from studies on DC biology in the design of novel vaccines.

2. Human DC subsets

2-1. Cutaneous DCs

Human skin hosts several distinct DC subsets. The epidermis hosts Langerhans cells (LCs), while the dermis displays two DC subsets, CD1a $^+$ DCs and CD14 $^+$ DCs, as well as macrophages [12]. Our studies on epidermal LCs and dermal CD14 $^+$ DCs revealed their phenotypical and functional differences. Dermal CD14 $^+$ DCs express a large number of C-type lectins including DC-SIGN (Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Non-integrin), DEC205, LOX-1 (Lectin-like oxidized LDL receptor-1), CLEC-6, Dectin-1 and DCIR (dendritic cell immunoreceptor), while LCs express Langerin and DCIR. Dermal CD14 $^+$ DCs express Toll-like receptors (TLRs) recognizing bacterial pathogen-associated molecular patterns (PAMPs), such as TLR2, 4, 5, 6, 8, and 10 [13,14]. LCs have been reported to express TLR1, 2, 3, 6, and 10 [13,15].

LCs and CD14 $^+$ DCs produce different sets of cytokines upon stimulation via CD40. CD14 $^+$ DCs produce a large set of soluble factors including IL-1 β , IL-6, IL-8, IL-10, IL-12, GM-CSF, MCP and TGF- β , while LCs produce only a few cytokines, including IL-15 [16]. Such different cytokine production profiles, at least partly, appear to explain why the two subsets induce adaptive immune responses with different qualities, as discussed hereunder.

2.1.1. CD4 $^+$ T cell responses—CD14 $^+$ DCs were originally found, using cells derived from CD34 $^+$ hematopoietic progenitor cells (HPCs), to help B cell responses through direct interactions. CD14 $^+$ DCs, but not LCs, induce CD40-activated naïve B cells to differentiate into IgM-producing plasma cells through the secretion of IL-6 and IL-12 [17]. Our recent studies indicate that CD14 $^+$ DCs induce naïve CD4 $^+$ T cells to differentiate into cells sharing properties with T follicular helper cells (Tfh) [16], a CD4 $^+$ T cell subset that controls B cell responses [18,19] (Figure 1). CD4 $^+$ T cells primed by CD14 $^+$ DCs (both generated *in vitro* and isolated from the skin) induce naïve B cells to proliferate and differentiate into antibody-secreting cells. Tfh cells, which express CXCR5, secrete IL-21 at higher levels than canonical Th subsets, including Th1, Th2, and Th17 cells [20-23]. IL-21 induces the growth, differentiation, and isotype switching of B cells [24,25], and plays a critical role in the development of germinal center responses [26,27]. In humans, DCs induce naïve CD4 $^+$ T cells to express IL-21 through the secretion of IL-12 which triggers STAT4 activation [28,29]. *In vitro*, IL-6, IL-23, and type I interferon can also induce naïve CD4 $^+$ T cells to express IL-21, though at a lesser extent [28-30]. IL-12 further promotes human CD4 $^+$ T cells to express ICOS, CXCR5, and Bcl-6 [28,29], molecules expressed by Tfh cells. Studies on mice revealed that STAT4 targets the *Il21* gene [31], though IL-12 does not induce mouse CD4 $^+$ T cells to express IL-21 [32,33]. Whether STAT4 signaling is associated to the development of Tfh cells in mice remains to be established.

LCs induce naïve CD4 $^+$ T cells to secrete Type 2 cytokines such as IL-4, IL-5 and IL-13, in addition to IFN- γ . This is consistent with mouse studies showing the preferential induction of Th2 responses upon delivery of an antigen to the LC-rich epidermis [34]. Others recently

demonstrated that human LCs promote the development of IL-22-secreting CD4⁺ T cells, which do not co-express Th1, Th2 or Th17 cytokines [35]. Such cells, named Th22 cells, are proposed to play pathogenic roles in skin diseases, such as psoriasis [36].

2.1.2. CD8⁺ T cell response—Our studies have shown that LCs are remarkably efficient at inducing cytotoxic T lymphocyte (CTL) responses. LCs, both generated *in vitro* from CD34⁺ HPCs and isolated from human epidermis, induce a robust proliferation of naïve allogeneic CD8⁺ T cells when compared to CD14⁺ DCs [16]. Upon loading with tumor-derived peptides, LCs efficiently prime peptide-specific naïve CD8⁺ T cells, and induce their differentiation into CTLs that express high levels of cytotoxic molecules and are accordingly highly efficient at killing tumor cells [16]. LCs are also efficient in cross-presenting peptides from protein antigens to CD8⁺ T cells [16,37]. While IL-12 [38] and IFN- α [39] are implicated in the induction of CTL responses by DCs, LCs do not secrete either cytokine upon stimulation with CD40 or TLR stimulation [15,16,40,41]. Instead, activated LCs secrete IL-15 [16,41], which we surmise responsible for their capacity to induce potent CTL responses. This hypothesis is partly supported by the observation that externally added IL-15 enhances the ability of CD14⁺ dermal DCs to develop CTLs with high levels of cytotoxic granules expression [14].

In contrast, several mouse studies, for example models using herpes simplex virus (HSV), have questioned the contribution of LCs to the induction of antigen-specific responses *in vivo*. These studies attribute the HSV-specific immunity to CD8 α ⁺ DCs, rather than to LCs [42]. Further *ex vivo* studies showed that dermal CD103⁺ DCs, but not dermal CD11b⁺ nor LCs, were able to present antigens to naïve TCR-transgenic CD8⁺ T cells *ex vivo* [43]. However, it remains to be determined whether these differences with regard to the function of LCs between mice and humans derive from the differences in their immune systems.

2.2 Blood DC subsets

Three subsets of DCs can be identified in human blood by the differential expression of cell surface molecules. CD1c⁺ (BDCA-1) DCs represent a major population of Lin^{neg}HLA-DR⁺CD11c⁺ DCs. CD1c⁺ DCs express a wide repertoire of TLRs, including TLR1, 2, 3, 4, 5, 6, 7, 8, and 10, and upon stimulation with TLR ligands they produce proinflammatory cytokines [44,45]. CD141⁺ (thrombomodulin, BDCA-3) Lin^{neg}HLA-DR⁺CD11c⁺ DCs [46] represent a minute population that uniquely expresses CLEC9A (also known as DNGR-1), a C-type lectin with ITAM-like motif [47]. Recent reports suggest that CD141⁺ DCs might represent the human counterpart of mouse CD8 α ⁺ DCs [48-52], a DC subset associated to the induction of CTL responses through their capacity to cross-present antigens [53]. Human CD141⁺ DCs, which do not express CD8, share with mouse CD8 α ⁺ DCs the expression of several surface molecules, including CLEC9A [48,50,54,55], the adhesion molecule NECL2 [50,56], and the chemokine receptor XCR1 [49,51]. Furthermore, similar to mouse CD8 α ⁺ DCs, human CD141⁺ DCs express TLR3 and TLR8, and upon stimulation with their ligands, efficiently induce CD8⁺ T cell responses through antigen cross-presentation [48-52]. While CD141⁺ DCs constitute less than 5% of blood DCs, they are also present in secondary lymphoid organs, such as spleen and tonsils [48-52].

The identification of human CD141⁺ DCs as the counterpart of mouse CD8 α ⁺ DCs opens the possibility to translate the knowledge generated in the mouse into humans. One should, however, translate mouse data into clinical applications with a critical mind, because 65 million years of independent evolution have brought in many nuances that distinguish the human and the mouse immune systems [57]. For example, other human DCs such as blood CD1c⁺ DCs [48,49,51] and epidermal LCs [37] can also cross-present antigens. Thus, it

remains to be determined whether and how CD141⁺ blood DCs are related to other DCs subsets and how all those DC subsets cooperate in shaping the adaptive immunity.

A third blood DC subset, plasmacytoid DCs [58] circulate in the blood and enter lymphoid organs through high endothelial venules (HEV) [59]. pDCs express high levels of IL-3R α chain (CD123), as well as some specific markers such as BDCA2, and ILT7 [60]. Studies in mice indicate that resting pDCs are involved in the induction of tolerance [61-63]. Furthermore, pDCs activated with IL-3/CD40L induce *in vitro* IL-10-secreting regulatory CD4⁺ T cells [64] as well as suppressor CD8⁺ T cells through the expression of ICOS ligand [65]. However pDCs can also contribute to the induction of immunity. Upon recognition of viral components through TLR7 and/or TLR9, pDC become activated and secrete large amounts of Type I IFN [59]. The pDC presents three remarkable cell biological features to counteract viral infection: 1) an extensive ER compartment that facilitates high-capacity secretion of antiviral factors, including type I interferons; 2) an early endosomal compartment containing MHC class I molecules that appears to permit direct vesicular MHC class I loading for immediate activation of memory cytotoxic CD8⁺ T cells [66]; 3) a late endosomal compartment containing MHC class II molecules, similar to that found in cDCs, which facilitates viral antigen presentation to CD4⁺ T cells. Thus, in both the MHC class I and class II pathways, pDCs may permit a rapid initial response to viral infections by utilizing pre-synthesized stores of MHC class I and II molecules. Indeed, pDCs exposed to virus can launch T cell responses [66,67]. pDCs may also be involved in the generation of plasma cells and antibody responses [68]. There, two mechanisms are employed to amplify B cell responses: 1) type I IFN and IL-6 upon viral stimulation [68]; 2) CD70 expressed on pDCs upon activation with CpG [69]. Thus, strategies designed to prime pDCs may form the basis of a next generation of antiviral vaccines.

Human pDCs are composed of at least two subsets, distinguished by the expression of CD2 [70]. Both subsets secrete IFN- α and express the cytotoxic molecules Granzyme B and TRAIL. However, the CD2^{high} pDCs are more potent than the CD2^{low} pDCs to induce allogeneic T cell proliferation. These different functional properties of CD2^{high} pDCs and CD2^{low} pDCs are associated to distinct transcription profiles, differential secretion of IL12 p40 and with differential expression of co-stimulatory molecule CD80 upon activation. Additional studies will be necessary to understand the biological role of these two pDC subsets.

In humans, pDCs can be mobilized *in vivo* with cytokines such as Flt3 ligand and G-CSF [71-73], while other blood DC subsets are mobilized by Flt3L only. The differential mobilization of distinct DC subsets or DC precursors by these cytokines offers a novel strategy to manipulate immune responses in humans [71,74].

3. Targeting dendritic cells for improved vaccines

Microbiologists, spearheaded by Louis Pasteur, have devised ways to generate vaccines by inactivating pathogens. Most of these vaccines act through the induction of humoral responses [75]. However, there are still many pathogens, for which no efficient vaccines are available, including HIV, Hepatitis C virus, Mycobacteria, Chlamydia, and Plasmodium, a parasite causing malaria. Most of these agents cause chronic diseases where strong cellular immunity, in particular CTL response, is critical for the clearance of the pathogens.

Studies on the functional characterizations of human DC subsets provide an opportunity for the novel design of therapies and vaccines. DC-based vaccines include two main approaches: *ex-vivo* generated DC vaccines and DC targeting. We will discuss here how DC subsets can be harnessed in a DC-targeting approach. For further details on *ex-vivo* generated DC vaccines the readers are encouraged to further read detailed reviews [76,77].

DC targeting represents a vaccine approach to deliver antigens directly to DCs *in vivo* using chimeric proteins composed of an anti-DC receptor antibody and an antigen (Figure 2). Pioneering studies by Michel Nussenzweig and Ralph Steinman with antibodies directed to DEC205 demonstrated that *in vivo* DC targeting results in a potent induction of antigen-specific CD4⁺ and CD8⁺ T cell responses in mice, provided adjuvants are co-administered to activate the targeted DCs [78,79]. The same group also elegantly proved *in vivo*, by using the DC-targeting approach, the concept of differential regulation of T cell immunity by distinct DC subsets [11]. There, antigens were selectively loaded *in vivo* onto distinct DC subsets, CD8α⁺ DCs expressing DEC205, or CD8α⁻ DCs expressing DCIR2, by using specific antibodies conjugated with OVA. CD8α⁺ DCs preferentially induce CD8⁺ T cell immunity, while CD8α⁻ DCs preferentially induce CD4⁺ T cell immunity. Accordingly, CD8α⁻ DCs and CD8α⁺ DCs preferentially express distinct sets of genes involved in MHC class II and class I presentation, respectively [11]. Furthermore, these subsets utilize different mechanisms for the induction of Th1 response. The DEC205⁺DCIR2⁻CD8⁺ DCs prime T cells to make IFN-γ in an IL-12-independent CD70-dependent fashion, while the DEC205⁻DCIR2⁺CD8⁻ DCs prime T cells in an IL-12-dependent fashion [80].

Our own studies with human skin DC subsets suggest that LCs are a target to consider for the induction of potent antigen-specific CTL response. Indeed, LCs targeted with a fusion protein composed of anti-DCIR antibody conjugated with antigens efficiently cross-present antigens to CD8⁺ T cells, and induce their proliferation *in vitro* [37]. Similar finding was also obtained when LCs were targeted with anti-Langerin antibody [37]. Furthermore, mouse studies demonstrated that injection of anti-Langerin chimeric proteins induce antigen-specific CD4⁺ and CD8⁺ T cell responses *in vivo* [81]. These observations provide a rationale that LCs can be utilized as a target in DC-targeting aiming at induction of CTL responses. In contrast, dermal CD14⁺ DCs might represent the appropriate target for the induction of potent humoral response. There, LOX-1 and DC-SIGN expressed by this subset may serve as the target DC receptors. Supporting evidence is that delivering antigens to DCs via DC-SIGN can elicit CD4⁺ and CD8⁺ T cell responses both *in vitro* [82] and *in vivo* [83,84]. CLEC9A may represent an interesting target to reach CD141⁺ DCs. Indeed, mouse studies showed that targeting antigen to CLEC9A expressed by CD8α⁺ DCs *in vivo* results in potent cytotoxic T lymphocyte responses when combined with anti-CD40 administration [54], and potent antibody responses even without co-administration of adjuvants [55].

In DC-targeting approach, selection of the appropriate adjuvant is a critical parameter for the induction of the immunity of the desired type (Figure 2). For example, although TLR-ligands are widely considered to promote protective immunity against infectious agents, selecting the appropriate ligand will be critical. For instance, TLR2 ligation, which promotes the induction of Treg cells rather than Th1 or Th17 cells [85], does not appear to be a preferred option in vaccines for cancer or infectious diseases. Our studies suggest that DCs exposed *in vitro* to anti-DCIR fusion protein induce different types of antigen-specific CD8⁺ T cell responses, when activated through different pathways. Thus, CD8⁺ T cells primed by DCIR-targeted DCs (generated by culturing monocytes with GM-CSF and IFN-α) activated through CD40 secrete Type 2 cytokines (IL-4, IL-5, and IL-13) together with IFN-γ, while those primed by DCs activated through TLR7/8 secrete no Type 2 cytokines, but higher levels of IFN-γ, and express more Granzymes and perforin [37].

Furthermore, certain lectins, for example Dectin-1 delivers activation signals to DCs. Dectin-1 recognize components expressed by fungi and mycobacteria, and deliver activation signals [83-85] through phosphorylation of tyrosine residues within the cytoplasmic ITAM motifs [86-89]. Syk and Card9 are recruited to the ITAM motifs [90-93], which leads to the activation of downstream molecules such as NFAT [94], MAPK [95], and NF-κB [88,90,96-99]. DCs stimulated through Dectin-1 secrete multiple cytokines, including IL-10,

IL-6, TNF α , and IL-23 [91,97,100], which leads to the induction and expansion of Th17 cell responses [90,91]. Targeting antigen to DCs via Dectin-1 generated strong CD4⁺ T cell responses, but weak CD8⁺ T cell responses in vivo in mice [101]. In contrast, our recent study shows that human monocyte-derived DCs incubated with recombinant proteins of an agonistic anti-Dectin-1 fused to antigens can elicit potent antigen-specific CD8⁺ T cell responses in vitro [102]. DC-SIGN is another lectin that can deliver intracellular signals [103-107]. DC-SIGN triggering activates RAF1, which induces the phosphorylation of the NF- κ B subunit p65 in a TLR-independent manner [107]. Thus, the challenge is to match the molecular target on DCs with the desired immune outcome, mimicking in many ways the natural role of these DC receptors to fine tune responses appropriate to the infection.

Another consideration is the localization of the target DCs and the tropism of the T cells activated by the DCs. DCs originating from a specific tissue have the capacity to instruct T cells to home back to that tissue [108]. Furthermore, DCs activated by different adjuvants might induce T cells with different migration properties. As optimal sites for T cell migration likely vary in different disease states, assessing this aspect is important for the design of vaccines. For example, whereas vaccines against cancer are expected to induce T cells that migrate into tumor sites, vaccines against influenza virus are desired to induce T cells to migrate into airway mucosal surfaces.

Therefore, multiple parameters need to be considered for the development of DC targeting vaccines (Figure 2). These include: 1) biological function of target DC subsets (for example, induction of humoral and/or cellular immunity), 2) the selection of antigens and their formulation to control the disease, 3) the receptors expressed by a given DC subset together with the choice of adjuvant, and 4) the tissue distribution of the target DCs. Further studies on the biology of human DC subsets will facilitate the design of efficient DC targeting approaches.

4. Concluding remarks

The progresses made in the knowledge of DC biology have opened the avenues for development of their clinical applications. This new knowledge represents a fertile ground for the development of novel approaches to intervene numerous clinical situations. Success of DC targeting in mouse models provide a rationale for potential human applications. The most efficient vaccines might actually be those that will target both CD14⁺ DCs and LCs, thereby allowing the maximal stimulation of both humoral and cellular immune responses. In this regard it is intriguing to consider that one of the most effective vaccines, smallpox vaccine, acts through a combination of strong cellular and humoral immunity and requires scarification of the skin, a procedure that injures both epidermis and dermis and that is likely to mobilize and activate LCs as well as dermal DCs. Likewise, one of the most potent vaccines ever generated against yellow fever (YF17D) activates multiple DC subsets [109] and leads to integrated immune response that includes both humoral and cellular immunity [110]. Further studies on human DC biology and in vivo testing using non-human primate models will facilitate the design of this novel type of vaccines aiming at potent antibody and/or CTL responses.

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Figure 1. Induction of distinct T cell responses by human cutaneous DC subsets

LCs are efficient at inducing high avidity-cytotoxic CD8⁺ T cell and Th2 and Th22 responses. In contrast, CD14⁺ dermal DCs are efficient at inducing antibody-secreting cells (ASC) and T follicular helper (Tfh) cells. Mouse studies demonstrate that activated dermal DCs migrate into the outer paracortex, just beneath the B cell follicles, whereas LCs migrate into the T cell rich inner paracortex [111]. These observations suggest that humoral immunity is preferentially regulated by CD14⁺ dermal DCs, while cellular immunity is preferentially regulated by LCs. GC B: germinal center B cells.

Figure 2. Strategy to modulate the quality of immune responses through DC targeting

DC targeting vaccines constitute anti-DC antibody conjugated with antigens, and potentially with adjuvants. Each component represents a different parameter. First, target antigens on DCs can be strategically selected based on the biology of the DC subsets that one anticipates to deliver antigens. Another consideration is the type of activation signals that the DC molecules may deliver. Second, selection of antigens and their formulation are the key to induce anticipated antigen-specific immune responses. Third, adjuvants can be selected based on the receptors that the target DC subset expresses, and the type of immune responses that one anticipates to induce. Lastly, the localization of the target DCs should be considered to induce T cells homing to the desired sites. Altogether, DC targeting has a potential to selectively induce the desired types of specific immune responses by strategically selecting these parameters.