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Engineering Immunity: Modulating Dendritic Cell Subsets and Lymph Node Response to Direct Immune-polarization and Vaccine Efficacy

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Abstract

While successful vaccines have been developed against many pathogens, there are still many diseases and pathogenic infections that are highly evasive to current vaccination strategies. Thus, more sophisticated approaches to control the type and quality of vaccine-induced immune response must be developed. Dendritic cells (DCs) are the sentinels of the body and play a critical role in immune response generation and direction by bridging innate and adaptive immunity. It is now well recognized that DCs can be separated into many subgroups, each of which has a unique function. Better understanding of how various DC subsets, in lymphoid organs and in the periphery, can be targeted through controlled delivery; and how these subsets modulate and control the resulting immune response could greatly enhance our ability to develop new, effective vaccines against complex diseases. In this review, we provide an overview of DC subset biology and discuss current immunotherapeutic strategies that utilize DC targeting to modulate and control immune responses.

Introduction

Vaccination has been the most effective public health strategy to control, and in some cases achieve, global eradication of infectious diseases. However, the full potential of vaccines is far from realized. Patients affected by devastating diseases, whether infectious (e.g. HIV, dengue virus and other emerging pathogens), endogenous (e.g. cancer or diabetes) or behavioral (e.g. drug addiction), are candidates for new vaccines and immunotherapies; but developing effective vaccines against these diseases have proven extremely challenging. Therefore, new approaches to (a) mount robust and sustained immune responses and (b) finely control the immune polarization to specific phenotypes that are therapeutic or protective for the specific condition, are critically needed. Investigation of more potent antigen and adjuvant combinations, incorporation of “smart” delivery vehicles, optimization of administration route and technique, and targeting specific cell types in the innate and adaptive immune system, are a few of the strategies being explored to achieve this.

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It is known that lymphoid organs, especially lymph nodes are hubs for immune cell interaction and play an indispensable role in providing an environment suitable for generation and maturation of the adaptive immune response. The classical adaptive response is initiated by antigen presenting cells (APCs) that encountered foreign and/or pathogenic material in peripheral tissues and migrated through lymphatic circulation to present antigen to T cells in the draining lymph nodes. Professional APCs encompass mainly dendritic cells (DCs) and macrophages in the periphery and it is now thought that DCs are the primary APCs responsible for signaling and directing T cell activity. Furthermore, it is now recognized that based on primary location, DCs can be sub-categorized into many functionally distinct groups, extending the influence that DCs have on immunity. There is a significant body of literature dedicated to vaccine design with peripheral DC activation, migration and antigen presentation in mind.

In addition to DC subsets in the periphery, there are also lymphoid-resident DC subsets that have significant impact on T cell maturation [1–3]. This discovery has sparked new research focused on targeting vaccine components directly to lymph nodes through the lymphatic vasculature or through systemic delivery. While others have investigated direct delivery to the lymph node using intranodal injection, we believe that this approach may be unnecessarily invasive, and will not be discussed in this review. When designing vehicles and approaches to target direct lymph node delivery, it is essential to keep in mind interstitial and lymphatic physiology and how this plays a role in regulating transport to the lymph nodes. These parameters are nicely highlighted in recent reviews by Thomas et al. and Swartz et al. [4,5], and readers are referred to those for further detail. In this review, we will focus on our current knowledge of DC subset biology and provide an investigative comparison between vaccine strategies targeting peripheral (i.e. skin) or lymphoid-resident DCs, including their major benefits and disadvantages as well as how these findings should shape vaccine design. Since most vaccines currently available and in development are administered via the subcutaneous or intramuscular routes, we will focus on delivery through these routes. In addition, commonalities and differences between murine and human DC subsets and related immune responses are also indicated, when known.

DC Subsets: An Overview

Based on our most recent knowledge of DC biology, there are several anatomically and functionally distinct DC subsets in peripheral tissues. In this section, we will discuss some of the key subsets and their functional differences. Table 1 provides a summary of these DC subsets, along with surface markers used to distinguish and isolate them, the related maturation markers and primary cytokine types that the cells secrete upon activation and maturation.

Additionally, Figure 1 delineates the skin, lymphatics, and lymph node biointerface; designating the relevant anatomy, various DC subsets present, and highlighting that peripheral DCs must migrate to the local immune hubs (i.e. draining lymph nodes) in order to initiate a robust adaptive, systemic immune response.

Secondary Lymphoid Organ Resident Dendritic Cells

Secondary lymphoid organ resident dendritic cells were the first to be classified in the mouse system by Steinman and Cohn over 40 years ago [28]. Since their initial discovery and characterization, our understanding of their complexity has greatly increased, leading to multiple sub-classifications with very different functions. To date, these DCs are placed into two subsets based on their expression of CD8 α and CD11b and their corresponding functions or in a third subset, which is the plasmacytoid DC.

CD8 α +DC

CD8 α +DCs, also characterized by their expression of C-type lectin receptor DEC-205 (CD205) but not integrin CD11b, have been the most extensively studied and shown to efficiently cross-present antigen, induce CD8+ T cell activity and cytotoxic behavior [1,2,6]. A functionally homologous cell type was only recently discovered in the human system and is characterized by its expression of BDCA-3 (discussed later in this section) [29,30]. It has also been shown that CD8 α + DCs have the ability to receive antigen from migratory DCs that arrive from the peripheral tissues for presentation to CD8+ T cells, effectively increasing the range of T cell activation associated with relatively few migratory cells in the LNs [31]. This antigen exchange may not be isolated to proteins, but may also include glycolipid antigens essential for natural killer-T cell activation [32]. Additionally, CD8 α + DCs play a role in maintaining self-tolerance by promoting self-reactive T cell proliferation and subsequent elimination, as well as maturation of Foxp3+ regulatory T (Treg) cells [3,33].

CD8 α - (CD11b+) DC

CD11b+ lymphoid DCs do not express CD8 α nor high levels of DEC-205, but can express CD4 and interact primarily with CD4+ T cells [2,8]. These cells preferentially activate a type II helper response from CD4+ T cells, likely due to their proficiency in MHC II antigen presentation [34–36]. These cells also exist in a double negative (CD8-CD4-) state and maintain their ability to promote type II polarization of the helper T cell (Th) response. However, CD4+ DCs have also been shown to secrete type I associated cytokines, such as IL-12, when activated by certain toll-like receptor (TLR) agonists, linking these cells to antiviral immunity with the appropriate agonists [37]. Lastly, these cells are the predominant scavengers of antigen that circulate through the lymphoid organs, especially lymph nodes, via lymphatic drainage [36].

Plasmacytoid DC

The final subset of lymphoid-resident dendritic cells is plasmacytoid DC (pDC), which has been identified in secondary lymphoid tissues as well as blood and certain peripheral tissues (mostly during inflammatory episodes) [38,39]. pDCs have been shown to differ from conventional DCs in morphology, migration patterns, and primary function [40,41]. Their hallmark is the ability to secrete large amounts of type I interferon after exposure to viral pathogenic patterns, particularly agonists for TLRs 7 and 9 [9,10,42]. These receptors typically recognize pathogenic RNA and DNA, respectively, and have mechanisms by which

they differentiate these patterns from autogenic nucleic acid debris [43]. However, there is recent evidence that certain disease states can promote recognition of self-DNA, leading to overproduction of type I interferon and autoimmunity [44–46]. pDCs have also been implicated in Treg priming, plasma cell differentiation and tolerance against allergies [47–51].

Skin-resident Dendritic Cell Subsets

Though peripheral dendritic cells reside in many organs such as the gut, intestine, and lungs, skin and muscle are the most common sites of vaccination. Because of recently developed devices such as microneedles and needle free injectors, skin-based vaccination is gaining further importance. Furthermore, significant knowledge exists on skin-resident DCs, their various subsets, and related lymphatic transport; which will be the primary focus of this section. It should be noted that that skin-resident DCs are likely responsible for patrolling skeletal muscle tissue as well, especially inflamed tissue, and therefore there is significant subset overlap [52,53].

Langerhans cells

Langerhans cells (LCs) are epidermal DCs characterized by their Birbeck granules, formed by the C-type lectin receptor, Langerin [13,54]. In addition, LCs express intermediate levels of CD11b, high levels of adhesion molecule EpCAM, and DEC-205 [14,17]. They specialize in surveying the epidermis for antigen, extending their dendrites between apical cells [55]. In mice, LCs (mLCs) can promote and regulate multiple T cell-mediated responses [26,56,57]. They primarily present antigen via MHC II to induce proliferation of Th17 cells, known for mediating immunity against bacteria and fungi, and Th2 cells, required for humoral immunity against large extracellular pathogens [14,15]. However, the ability of mLCs to induce type I helper immunity is controversial. In mice infected with *Candida albicans*, mLCs were unable to induce Th1 responses or cross-present antigens to CD8+ T cells [15]. However, Nizza et al. observed that murine CD11b+ Langerin+ migratory DCs (mainly mLCs) were able to cross-prime naïve CD8+ T cells and imprint them with skin-homing specificity [16]. mLCs can also induce proliferation of Treg cells and restrain self-reactive T cells, suppressing inflammation and autoimmunity [14].

The discovery by Nizza et al. reveals that mLCs may be more equivalent to human LCs (hLCs) than previously speculated. Like mLCs, hLCs express Langerin and EpCAM in addition to CD1a. However in humans, expression of DEC-205 may be inflammation-dependent [22,26]. hLCs are highly efficient at cross-presenting antigen to cytotoxic T cells [19,26,58]. They are more efficient than dermal DCs at priming naïve CD4+ and CD8+ T cells, possibly due to IL-15 secretion as opposed to IL-10 [59]. hLCs can also drive proliferation of epidermal Tregs in the steady state, but limit their activation during inflammation [60].

Dermal Dendritic Cells

A basement membrane separates the epidermis from the dermis, a layer primarily composed of fibroblasts, collagen, and elastin fibers [61]. Within the dermis are conventional DCs

(cDCs), distinguishable from their lymphoid-resident counterparts by their intermediate to high levels of CD11c and high levels of MHC II [62,63]. In both homeostatic and inflammatory conditions, they travel from the skin to lymph node guided by the chemokine receptor, CCR7, which will be discussed in greater detail later. At steady state, dermal DCs present self-antigens, aiding in maintenance of peripheral tolerance. However, when they encounter a pathogen, dermal DCs mature similarly to LCs and upregulate costimulatory molecules on their surface in order to activate antigen-specific CD4⁺ and CD8⁺ T cells. The two major murine dermal DC subsets are CD103⁺ and CD11b⁺ DCs [55,64].

In the dermis, CD103⁺ DCs express Langerin but not CD11b (although some are CD11b^{lo}) [17,55]. These dermal DCs are most known for their superior ability to cross-prime CD8⁺ T cells [18]. Waithman et al. found CD8⁺ T cell responses were severely hindered in influenza infected C57BL/6 mice deficient in transcription factor Batf3, which is required for the development of CD103⁺ DCs and their functional lymphoid-resident counterparts, CD8α⁺ DCs [17,65]. Both subtypes express Clec9A, TLR3, and XCR1, necessary for sensing necrotic bodies, viral dsRNA, and promoting differentiation of CTLs, respectively [27,61]. In addition to cross-presentation, CD103⁺ DCs also drive Th1 and Th17 cell differentiation [15,66].

CD11b⁺ DCs express DEC-205, but not Langerin [17,55,63]. They are the most abundant type of DC in a healthy dermis and share phenotypical characteristics with monocyte-derived DCs that infiltrate tissue during inflammation [20,61]. They can also be broken down into further subcategories with varying functions but their predominant role is thought to be MHC II presentation and induction of Treg and Th cells [67,68]. For example, Plantinga et al. observed that migratory CD11b⁺ cDCs but not CD103⁺ DCs initiated Th2 cell-mediated immunity against house dust mite allergen in the lymph node [20]. Though they do not directly activate Treg cells, they express the enzyme aldehyde dehydrogenase that metabolizes vitamin A into retinoic acid, which has been implicated in Foxp3⁺ Treg cell generation in vitro [21,69,70].

The two main DC subsets of the human dermis are CD1a⁺ and CD14⁺ cDCs [71,72]. CD1a⁺ DCs are able to stimulate CD4⁺ T cell proliferation and prime CD8⁺ T cells [22,23]. They prime CD8⁺ T cells better than CD14⁺ DCs, but less potently than LCs [58]. Upon maturation, they overexpress costimulatory molecule CD83, and higher levels of CCR7 than CD14⁺ DCs [73,74]. They also express lower levels of DEC205 than murine dermal DCs, and virtually no Langerin.

CD14⁺ DCs are macrophage-like cells that preferentially induce humoral immunity and are the murine homologue to a CD11b⁺ dermal subset [11,75]. Similar to the murine system, they promote type II polarization of CD4⁺ T cells which causes B cells to differentiate into plasma cells [24,25,58]. They poorly cross-present antigen, but have been shown to activate CD8⁺ T cells if antigen is internalized via DCSIGN [22]. Angel et al. claims that DCSIGN is restricted to this subset in the human system, along with TLR2 and macrophage mannose receptor (CD206) [76]. CD14⁺ dermal DCs generally exhibit immature features in comparison to CD1a⁺ DCs; however, they are capable of expressing CD83 while lowering

expression of TLR2 [76]. They also have limited migratory potential due to their lower expression of CCR7 in comparison to CD1a+ DCs [11,22,58,75].

Recently Haniffa et al. identified a DC subset distinct from CD1a+ and CD14+ DCs, referred to as CD141+BDCA-3 cross-presenting DCs [27]. They are functionally homologous to murine CD103+ dermal and CD8+ lymphoid DCs, (evident by their superior cross-presenting ability) and are migratory [11,26,27].

During inflammation, the immune cell makeup of tissue drastically changes due to the recruitment of granulocytes, classical monocytes and pDCs [11]. These immune cells infiltrate tissue in response to inflammatory stimuli, such as TNF-alpha and nitric oxide [14]. Human CD14+ Ly6C^{hi} classical monocytes are the supposed precursors of inflammatory DCs, characterized by the expression of Ly6C, CD11b, MHC II and intermediate CD11c levels in mice. Chemokine receptor CCR2 is also expressed on these cells and controls monocyte recruitment from bone marrow to the site of inflammation [77,78]. Upon tissue entry, inflammatory DCs down-regulate Ly6C, making them difficult to distinguish from steady state, skin-resident CD11b+ DCs [55,61]. Few pDCs are found in quiescent interstitial tissues, but have been found to promote wound repair in inflamed regions of the skin [11,12].

DC Migration

Migratory dendritic cells travel to the lymph node through afferent lymphatic vessels in homeostatic and inflammatory conditions. Tissue-resident dendritic cells are constantly presenting antigens; in the steady state, they present self-antigens via MHC II, resulting in tolerogenic activity. Immature migratory dendritic cells do not express high levels of co-stimulatory molecules such as CD40, CD80, or CD86, which bind to receptors on T cells during activation [79,80]. In the presence of MHC II without engagement of co-stimulatory molecules, T cells either die or become anergic [81]. Immature, migratory DCs can also convert naïve CD4+ T cells into Tregs [82].

It is well known that DCs activated by microbial antigens and inflammatory stimuli upregulate chemokine receptor CCR7, which binds to ligands CCL21/SLC and CCL19/ELC for recruitment to the lymphatic vessels [83–85]. CCL21 is released by lymphatic endothelial cells (LECs) and is highly concentrated around the vessel, since it binds to sulfated proteoglycans (i.e. heparin) [4,86]. On the other hand, CCL19 is more soluble and acts in both autocrine and paracrine fashions, as it is released by both dendritic cells and LECs [4]. While this recruiting mechanism may play a role in steady state maturation and migration, there is still much to learn. Baratin et al. speculates NF- κ B signaling is required for maturation and migration and demonstrates IKK β deletion is sufficient to prevent the steady state accumulation of migratory DCs in the lymph node [87].

Targeting Strategies

A common approach to modulating immunity and control immune-polarization is targeting DCs, the conductors of the resultant adaptive response. As discussed previously, there are DCs in multiple locations; therefore targeting techniques vary based on which subset is of

interest. This section will explore multiple approaches to targeting both skin and lymph node resident DCs. Furthermore, we discuss advantages and disadvantages of each technique. Table 2 summarizes the targeting strategies discussed in this section.

Skin Dendritic Cell Targeting

The route in which a vaccine is administered is known to affect resultant immunity and therefore is an important aspect of vaccine strategy [116]. Mucosal regions, such as the gut, lungs and reproductive tract, are known to have significant populations of DCs and are therefore important regions of interest for vaccination. However, those studies have been extensively reviewed previously and therefore, will not be the focus of this section [117–119]. While the intravenous (IV) route has not been extensively studied for vaccine delivery, a few investigators have had success utilizing it. Pooley et al. was able to demonstrate that soluble antigen delivered IV induced presentation to both CD4+ and CD8+ T cells by resident DC populations in the lymph nodes [2]. Seder et al. also achieved protection against malaria infection in humans after IV administration of an anti-malaria vaccination [120]. However, administration of vaccine directly into the blood has limitations, including fast clearance, non-specific interactions with other cell types exposed, systemic toxicity related to potentially massive cytokine release, and constraints on carrier parameters, such as size. Because of these factors, the most common route of administration targets the many subsets of DCs in the skin, which are responsible for patrolling epidermal, dermal and muscle tissues.

The most common approach to targeting skin DCs is modification of vaccine components/carriers to promote direct interaction with specific DC subsets of interest. As discussed previously for lymph node resident DC targeting, C-type lectin receptors (CLRs) are a particularly popular choice for skin DC targeting as well [121]. These include DEC-205, DC-SIGN and other mannose receptors. DEC-205 has been used to preferentially deliver formulations to promote both immunity and tolerance [122,123]. This difference in response is dictated by the antigen that is delivered by the DEC-205 targeted carrier. At steady state, tissue-resident DCs promote tolerance to self-antigens; therefore in autoimmune diseases tolerogenic DC phenotypes can be promoted by targeting appropriate antigens and immune-modulators using DEC-205. On the other hand “foreign” antigens and strong, immune-stimulatory adjuvants targeted to DCs via DEC-205 can promote vaccine responses. Kwon et al., using DEC-205 antibody targeting on a pH responsive carrier was able to improve migration of DCs to draining lymph nodes and subsequent interferon- γ production by activated T cells [122]. Inversely, Spiering et al. used the same targeting approach to deliver an immunodominant peptide to DCs, inducing tolerance and combating arthritis [123]. DC-SIGN has been used similarly as a choice receptor for DC targeting and also promotes rapid internalization and presentation to CD4+ T cells [89]. Going along the same lines, many others have investigated modifying carriers with glycans that are ligands for highly expressed CLRs and successfully demonstrated increased activation of both CD8+ and CD4+ T cells [22,92,93]. Furthermore, mannose receptor ligands, such as mannosylated alginate, have proven to be an effective means of targeting skin DCs as well [95]. Another technique that utilizes specific CLR expression on DCs targets DC-SIGN via a lentiviral vector. This vector simultaneously was able to transduce DCs, inducing maturation and

promote efficient antigen presentation [94] Interestingly, this has also been shown effective when the lentiviral vector binds DC MHC II [124].

While these strategies are effective, they do require potentially expensive antibodies or other targeting molecules. It has recently been hypothesized that physical parameters, such as carrier size or shape, can potentially be tuned to target different subsets of DCs. While the effect of size on DC uptake and maturation has been studied extensively, no definitive conclusions about targeting DC subsets have been reached. However, it has been shown that different subsets, mainly conventional vs. plasmacytoid DCs, do exhibit different phagocytic behavior [105], informing the hypothesis that it's possible that they would respond differently to carriers of varying sizes. Carrier charge could also play a role in preferential DC subset targeting. It has been observed that cationic particles are able to trigger TLR4 signaling [106]. Therefore, it's possible that DC subsets with higher expression of TLR4 may become activated more rapidly and robustly by cationic carriers, promoting their migration to lymph nodes. Lastly, while carrier geometry has not been studied in the context of DC subset targeting, it has been studied in other cell subsets. In fact, Agarwal et al. was able to demonstrate that bone marrow derived DCs did preferentially take up nanodiscs over nanorods in vitro [107]. These studies confirm the potential for studying the potential for using physical carrier parameters to target desired immune cells, including DC subsets.

Because the restrictions on size, shape, etc. are much broader for vaccine formulations meant to remain at the peripheral site of injection, many studies have developed immune priming depots to promote local DC migration and prolonged antigen/adjuvant delivery. Depot systems can either be implantable or in situ forming and therefore, injectable. Hydrogels are typically used in in situ systems, but large particles that cannot benefit from lymphatic drainage can also form a “depot” at the injection site. These depot systems have been designed to deliver a variety of vaccine components including cells, growth factors and particulate antigen/adjuvant carriers. Hori et al. directly delivered in situ activated DCs in alginate gels, promoting endogenous DC migration to the depot and leading to amplification of the immune response [97]. These in situ forming gels relied on the co-delivery of calcium microsphere reservoirs to induce gelation, which could also be loaded with adjuvant and/or cytokine to direct immunity [98]. A similar depot system has been described by Singh et al., employing thermo-sensitive chemistry for in situ gelation. They were able to deliver a DC chemoattractant alongside polymeric vaccine carriers that were easily taken up by local DCs, resulting in enhanced protection against B cell lymphoma [99–101]. Self-assembling peptide constructs are also being used to form high-aspect ratio, self-adjuvanting beta sheets [102]. This platform allows precise control over epitope loading ratio, providing a means of discretely tuning T and B cell responses in vivo without nonspecific inflammation [103]. Inorganic scaffolds have also been used as a means to a similar end. High-aspect-ratio mesoporous silica rods, when injected subcutaneously, form a porous structure that allows for skin DC migration and activation [104]. All of these approaches have improved one or multiple aspects of the DC arm of immune modulation, including antigen delivery, activation, migration and antigen presentation.

Lymph Node Targeting Strategies

It is now well appreciated that lymphoid resident DC populations have the ability to generate an immune response independent of peripheral migratory populations; therefore, they have inspired a new field of drug delivery research that involves targeting formulations to secondary lymphoid organs. In this review, we will focus primarily on those that are directed to lymph nodes. Researchers in the mid-1990s were already aware that lymph node targeting was advantageous, especially for vaccines against immune cell infection, such as the human immunodeficiency virus [125]. In these studies, Lehner et al. utilized strategic route of administration as a means of preferentially delivering subunit simian immunodeficiency virus (SIV) vaccine to iliac lymph nodes of infected macaques [126]. These researchers were benefiting from a simple phenomenon that many others have taken advantage of to date, which is the unidirectional flow of fluid from the interstitium to draining lymphatic vasculature, providing a one-way ticket to the lymph node [127]. Lymphatic flow and physiology have both been extensively reviewed recently [4,5,128]; therefore this section will focus on vaccine design strategies and applications.

Targeting techniques that take advantage of lymphatic drainage must consider design parameters including formulation size and surface properties, such as charge, hydrophobicity and chemical composition. Many studies have investigated optimal size for lymphatic drainage. The general consensus is that vaccine components are able to independently and efficiently drain into the lymphatic vasculature if they are under 100nm in diameter [108–111]. This has been found to be true for a large variety of delivery platforms including polymeric nanoparticles [108,110], liposomes [109] and virus-like particles [111]. However, in addition to accumulation in the lymph node, particles must also remain in the nodes in order to facilitate uptake and modulation of the resident immune cells. Oussoren et al. observed that smaller liposomes (~70nm) did efficiently collect in the draining lymph nodes but were then rapidly moved into circulation, limiting their efficacy [109]. However, more recent studies done with polymer particles have shown that 20-40nm particles are successfully retained within the lymph nodes [110]. This could be result of particle surface properties altering their behavior. For example, it has been shown that modification of particle surfaces with poly ethylene glycol can increase retention time [129]. Hanson et al. used PEGylation to improve collection of their liposome formulation in draining lymph nodes, allowing delivery of a potent adjuvant, cyclic-GMP specifically to resident APCs, followed by a significant improvement in immune activation, including expansion of CD8+ and CD4+ T cells as well as germinal center formation and antibody class switching [130].

Favorable trafficking and retention has also been demonstrated with particles that are cationic or decorated with targeting molecules, such as antibodies[131–134]. Targeting using antibodies against DEC-205 has proven to be an effective strategy, increasing MHC I associated antigen presentation on lymph node resident DCs 400 fold in one study [90]. This response was found to be enhanced even more with the addition of an agonist [135]. Cytotoxic immunity improvement has also been demonstrated using Langerin and Clec9A targeting antibodies[136]. Others have found that targeting other molecules, such as F4/80 like receptor and C-type lectin receptor, on CD8- DCs specifically, resulting in a robust antibody response[96].

Recently, other innovative techniques have been developed to target lymph node resident DC populations in attempts to enhance modulation of the immune response. Reddy et al. developed ultra-small polymer particles (25nm) that were chemically modified to activate the complement system upon injection. This activation technique resulted in efficient generation of a balanced cellular and humoral response against a model protein antigen [112]. The same particle platform carrying CpG as an agonist was also used as a cancer therapeutic [113]. Additionally, Thomas et al. demonstrated that by injecting the particles upstream of tumor draining lymph nodes that the particles were able to stimulate an anti-cancer immune response without delivery of an antigen [114]. Another intriguing technique involves self-assembly of peptide-based structures. Liu et al. developed an amphiphilic peptide construct with an albumin binding tail, enabling the vaccine to “hitchhike” onto endogenous albumin, which facilitated very efficient transport to the draining lymph nodes. With the addition of a TLR agonist, they demonstrated a 30-fold increase in T cell activation relative to soluble controls[115].

Comparison

Clearly there is a precedent for studying and designing vaccine formulations for optimal delivery to DC populations of interest. However, it is beneficial to think critically about the arguments for and against each of these approaches. For years, the case for peripheral vaccination was made based on an incorrect theory that lymphoid tissue resident DC populations were already in a mature state that prevented them from taking up and presenting new antigen. However, this has been emphatically disproved, opening the door to utilizing these cells for immune modulation [137]. Those who study lymphoid DC targeting contend that preferentially delivering antigen to lymphoid tissue allows access to a higher concentration of DCs, thereby inherently increasing the delivery of the vaccine to desired cells [138]. Additionally, the DCs are already homed very near T cell zones, allowing rapid antigen presentation and mounting of the immune response [139,140]. Lastly, some studies have proposed that peripheral DCs can become prematurely activated and present antigen prior to migration into the lymph nodes, inducing tolerance instead of immunity [141]. While these are all strong arguments for lymph tissue targeting, these techniques can also prove to be limiting. It has been proposed that carrier parameters (i.e. size, charge, shape, etc.) may all have a distinct effect on immunity [142–145]. However, due to the restrictions of transport in the interstitium, many of these parameters cannot be tested due to lack of drainage of larger or high aspect ratio particles [5,146]. However, these carriers are still accessible to peripheral DCs, which can migrate to the site of inoculation.

While most vaccine formulations are designed to target DCs in one particular tissue (i.e. skin, draining lymph node, etc.) it has been suggested in multiple studies that it may be most advantageous to target multiple populations simultaneously. It was discussed previously in this review that migratory DCs may transfer antigen to local DC populations in secondary lymphoid tissues, broadening their ability to stimulate many T cells [31]. Alternatively, Belz et al. observed that, in models of lung viral infection, that both migratory DCs and nasal lymphoid tissue contribute significantly to MHC I restricted antigen presentation. Therefore, both subsets are critical for virus elimination [147]. This supports a theory that perhaps only the combination of resident and migratory DCs provides the increase in the number of

antigen presenting cells at the site of T cell activation that is necessary for robust immunity. A later study also came to a similar conclusion for CD4⁺ T cell specific engagement and activation. Furthermore, they were able to elucidate a specific role for each cell type. They proposed that resident DCs, upon receiving antigenic signals, prevent T cell egress from draining lymph nodes, effectively “trapping” them in the region of efficient migratory DC antigen presentation [148]. This expands on the previously proposed theory and further clarifies subset specific responsibilities.

Finally, as was described earlier, it has been proposed multiple times in the literature that only particulate structures under ~100nm can efficiently drain into peripheral lymphatic vessels and traffic to the lymph node passively. Given this theory, it was thought that migratory DCs were the sole means of transporting larger antigens and/or carriers to the lymph node. A recent study published by Gerner et al. challenges this idea by using high-resolution whole lymph node imaging. Not only did they describe a novel DC subset that is responsible for rapid CD8⁺ and CD4⁺ T cell activation, but also confirmed that polymer particles as large as 1 micron in diameter were detectable in the lymphatic sinuses within minutes of delivery into skin (Figure 2) [36]. While this discovery does expand the importance of resident DC populations, the authors also observed that without migration of peripheral DCs, CD4⁺ T cell populations exhibited less cytokine activity and antibody titers were significantly decreased. Therefore, this detailed investigation presents another example of the interconnectivity between peripheral and resident DC populations and encourages the exploration of strategies to enhance both responses simultaneously.

Clinical Trials

As described in previous sections, there is extensive evidence demonstrating the efficacy of DC targeting as an immunomodulatory strategy for many therapeutic applications. Additionally, some of these strategies, particularly antibody targeting, have been translated into clinical trials, some of which have been successful. Table 3 lists examples of clinical trials that have utilized DC targeting strategies in applications ranging from cancer to anti-viral therapies [91]. The variability of success can likely be attributed to multiple factors. First, it is known that molecular targets of antibodies like DEC-205 and mannose receptor are not exclusively restricted to DCs in humans, but can also be found on monocytes and other lymphocytes in some cases. This dilutes the therapeutic effect and could have potential adverse non-specific effects. Therefore, investigation of more specific targets remains essential. This is also true for strategies that either target phagocytes in the skin using carrier parameters or preferentially drain into lymph nodes. Furthermore, the majority of applications for DC targeted therapies are incredibly immunologically complex, as is the case for the examples cited in Table 3 (i.e. cancer and HIV). It is becoming exceedingly clear that a single therapy may not be the answer to provide a robust treatment for these evasive diseases, but that combinatorial therapies will likely be necessary to combat them from multiple fronts. For example, a cancer immunotherapy like a DC targeted carrier would likely benefit from being combined with a strategy for minimizing immunosuppressive cells and signals, such as the PDL1, PD1 or CTLA-4 antibodies that were recently approved by the FDA [149].

Other APCs: Macrophage Targeting

While the most significant body of APC research focuses on DCs, targeting other APCs could be useful for select therapies. For example, macrophages populate tumors and can become tumor-associated macrophages, known for their immune-suppressive function [150]. However, the plasticity of macrophages provides an opportunity to utilize these cells for tumor cytotoxicity [151]. It can therefore be appreciated that these anti-tumor macrophages can be utilized for cancer immunotherapies. Indeed, Apostolopoulos et al. showed that mice that received a single infusion of ex vivo primed autogenic macrophages pulsed with an adenocarcinoma associated protein MUC1 had a CTL response similar to mice who received 3 injections of a soluble formulation of the vaccine [152]. Macrophages are also responsible for maintaining T cell tolerance to self-antigens [153]. They have therefore been used to eliminate autoreactive T cells in mice with experimental autoimmune encephalomyelitis (EAE) [154].

Techniques that have been presented above for DC targeting can easily be modified for macrophage targeting. Instead of antibodies specific for DEC-205 or DC-SIGN, macrophage specific molecules, like macrophage scavenger receptor (MSR) or sialic binding lectin CD169 can be using as targets instead [155,156]. It's been shown that CD169 antigen targeting can lead to a robust humoral response [157]. Additionally, it is likely that physical parameter (i.e. size, charge, shape, etc.) targeting described earlier can also be applied to macrophages.

Conclusion and Future Perspectives

Vaccines can be considered as the single most successful tool in human history to reduce mortality (especially infant and childhood mortality), increase global life-expectancy, and prevent debilitating diseases. Despite their tremendous success in many diseases, vaccination remains a holy grail for many others, including cancer, HIV/AIDS, emerging infectious diseases, addiction, etc. Further, beyond the realm of preventative vaccines, healthcare is currently on the precipice of embracing immunotherapy as one of the primary approaches for treating evasive and complex diseases. This is hallmarked by the FDA approval of a cellular immunotherapy for prostate cancer called Provenge (Dendreon) and more recently, an antibody therapy for metastatic melanoma, Keytruda (Merck). A large number of other immunotherapy approaches are currently in clinical trials and we will continue to see introduction of many such therapies into clinical practice in the very near future.

Many emerging vaccines and new immunotherapy strategies are designed to target and modulate DCs, the link between innate and adaptive immunity. These direct in vivo manipulation techniques benefit from being relatively easy to scale; especially compared to cell therapy approaches. Extensive studies are underway to develop the most efficient means of modulating DC behavior in vivo, and thereby control immune-polarization. However, there are still gaps in our knowledge that preclude our ability to produce therapies that are robustly effective for the vast majority of patients.

As mentioned previously, most current approaches of targeting DCs also affect other cell types. It remains a mystery which subset (or subsets) of immune cells are the most important to target for any given disease. It is likely that the answer to that will be simultaneous activation of multiple subsets, in a temporally and anatomically controlled manner; but we are far from understanding our own immune system with that level of granularity. In the coming years and decades, vaccines, immunotherapies and related delivery systems, must thrive to understand the underlying molecular signaling mechanisms by which subsets of DCs and other immune cell orchestrate systemic immunity and how to precisely modulate that process using controlled delivery approaches. It is expected that different delivery approaches (for the same antigen and adjuvant) will result in different signaling pattern (kinetics and strength) and can result in unique DC responses in terms of activation, cytokine profile, etc. But little is known in how carrier properties effect DC signaling.

Another key under-studied area in vaccine research is the issue of transport. Peripheral vaccines and adjuvants as well as the cells they effect, must travel to the local immune organs (e.g. draining lymph nodes, dLNs) to elicit an immune response. The spatio-temporal pattern of that transport process, i.e. how, where and when antigens, adjuvants and relevant DCs (and which subsets of DCs) are transported to dLNs and how that effect which cells in the dLNs they interact with, and what types of immune responses gets elicited, is of critical importance. Continuing with that concept, immune evasive disease like the ones being tackled by many of these therapies are particularly challenging and will likely require a combination of multiple techniques to provide long-lasting disease stabilization and/or remission; e.g. vaccination combined with immune-checkpoint blockade or tumor-microenvironment modulation. Little research has been done in such combinatorial approaches.

Concerted, engineering design-driven efforts, in close collaboration with immunologists and clinical scientists, that addresses these challenges, could fundamentally transform how we defend ourselves against deadly diseases in the future.

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Figure 1. Schematic of skin and lymph node resident DC subsets

(top) Lymphatic vasculature drains peripheral tissue such as the skin. Lymph flows unidirectionally through tissue and to draining lymph nodes. (Left) Skin resident DCs constantly survey the tissue for pathogenic or foreign material. While many of these cells are present in steady state tissue, others are found in appreciable numbers following inflammatory stimulus, such as pathogenic invasion, inflammation or injury. Following exposure and activation, these cells migrate into lymphatic vessels by following a chemokine gradient and then follow the lymphatics to draining lymph nodes. (Right) Draining lymph nodes are home to cells of the adaptive immune response, including T and B lymphocytes. Additionally, there are resident DCs, responsible for providing rapid signals to lymphocytes after antigen retrieval from the lymph. Migratory DCs also interact with these cells. Together, these potent DC signals provide the niche needed for appropriate immune-polarization and efficient generation of therapeutic and protective systemic immune responses.

Figure 2. Bacteria and micron-sized particles can rapidly drain to lymph nodes

Images from recent publication by Gerner et al [36]. (A) 1µm polystyrene beads were able to drain to skin draining lymph nodes of mice within 30 minutes of application. Additionally, these particles were specifically associated with lymph node resident DCs (green). (B) After administration of *P. aeruginosa* (red), bacteria were observed interacting with LN sinus resident DCs within 4 minutes of inoculation.

Table 1

DC Subset Biology

DC Subset	Primary Location	Species	Phenotype	Function [†]	Source
CD8a+	Lymphoid tissue	Mouse (M)	CD205 ⁺ CD11b ^{lo/-} MHC I Clec9A TLR 3	Cross presentation CD8+ T cell priming Maintain self-tolerance	[1,2,6,7]
CD8a-	Lymphoid tissue	M	CD205 ^{lo} CD11b ⁺ CD4 ^{+/-} MHC II TLR 7	CD4+ T cell activation	[2,7,8]
Plasmacytoid (pDC)	Blood/Lymphoid Tissue/Inflammatory Tissue	M/Human (H)	CD11c ^{lo} TLR 7 TLR 9	Type I interferon secretion Promote wound repair	[9–12]
Langherans	Epidermis	M/H	Langerin ⁺ CD205 ⁺ * CD11b ⁺ EpCAM ⁺ ** MHC I (M) MHC II (M) HLA-DR (H)	CD4+ T cell priming Th2/Th17 induction Treg induction Cross presentation **	[13–16]
CD103+	Dermis	M	Langerin ⁺ CD11b ^{lo/-} CD11c ⁺ MHC I MHC II Clec9A TLR 3	CD8+ T cell priming Cross presentation Th1/Th17 induction	[15,17–19]
CD11b+	Dermis	M	Langerin ⁺ CD11b ⁺ CD11c ⁺ MHC II	Treg induction Th induction	[17,20,21]
CD1a+	Dermis	H	Langerin ⁺ CD205 ⁺ CD11c ⁺ HLA-DR ⁺	CD8+ T cell priming CD4+ T cell proliferation	[22,23]
CD14+	Dermis	H	Langerin ⁺ CD11c ⁺ DCSIGN ⁺ CD206 ⁺ HLA-DR ⁺	CD4+ T cell activation Th2 induction	[24,24,25]

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DC Subset	Primary Location	Species	Phenotype	Function [‡]	Source
CD141+	Blood/Lymphoid Tissue/Dermis	H	BCDA3 ⁺ CD11c ⁺ CD1a ⁺ CD11b ^{lo} HLA-DR ⁺	Cross presentation	[11,26,27]
Mo-derived	Blood/Inflammatory Tissue	M/H	Ly6C ⁺ CD11b ⁺ CD11c ⁺ MHC II	Infiltrate inflammatory tissue	[11,14]

[‡] DC subsets not limited to these functions; those listed are discussed in this review

^{*} Expression of CD205 may be inflammation dependent in humans

^{**} Controversial whether mLC's cross present

Table 2

DC Targeting Strategies

Strategy	Cells Targeted	Results	Sources
Antibody targeting	DEC-205+ cells	<ul style="list-style-type: none"> - With pH responsive carrier, improved migration of DCs to lymph nodes - Delivery of immunodominant peptide to DCs, combating autoimmunity - Increased MHC I-dependent antigen presentation on lymphnode resident DCs - Active clinical trials 	[88–91]
	C-type lectin receptor (CLR)+ cells e.g. DC-SIGN	<ul style="list-style-type: none"> - Increased activation of CD8+ and CD4+ T cells - Promotes efficient DC activation 	[22,92–94]
	Mannose receptor (MR)+ cells	<ul style="list-style-type: none"> - Effective skin DC targeting - Active clinical trials 	[91,95]
	Other antibodies, e.g. Langerin, Clec9, F4/80	<ul style="list-style-type: none"> - Induces robust antibody response driven by lymph node resident DCs 	[69,96]
Carrier parameter targeting	Various phagocytic subsets	<ul style="list-style-type: none"> - DC subsets exhibit varying phagocytic behavior - DCs expressing higher amounts of TLR4 maybe be preferentially activated by cationic particles - Bone marrow derived DCs take up discoid carriers preferentially over rods - Carriers <100nm in diameter drain into lymph nodes, promoting activation of lymphnode resident DCs - Carriers ~25nm in size traffic to tumor draining lymph nodes, providing therapeutic value 	[97–104]
Immune depot	Various DCs	<ul style="list-style-type: none"> - Addition of DC specific chemokines and cytokines to promote DC activation and migration 	[105–114]
Albumin “hitchhiking”	Lymph node resident DCs	<ul style="list-style-type: none"> - Peptide binds to endogenous albumin, providing efficient transport to lymph node DCs, leading to a 30-fold increase in T cell activation over controls 	[115]

Table 3

DC Targeting – Clinical Trials

Phase	Years	Targeting Strategy	Indication	Results
I	1994-1997	Oxidised mannan fused with MUC-1 protein	Advanced metastatic adenocarcinoma	Half of patients had elevated antigen-specific IgG1. Some patients also had elevated T cell response.
I	1994-1997	Oxidised mannan – MUC-1	Cancer of breast, colon and other organs	60% generated robust IgG1 response, 28% exhibited cellular response.
III	1994-1997	Oxidised mannan – MUC-1	Breast cancer	2/16 reoccurrences with therapy versus 9/16 in control group
I/II	2004-2010	Mannose-receptor antibody fused with recombinant human chortonic gonadotropin beta-chain	Epithelial carcinoma	Best results after addition of GMCSF and TLR3/7/8 agonists
I	2009-2014	DEC-205 antibody fused with NY-ESO-1	Melanoma	Enhanced activation of CD8+ T cells
II	2014-present	NY-ESO-1 fused to DEC-205 antibody with TLR3/7/8 agonists	Advanced stage melanoma	13/45 patients demonstrated disease stabilization and 2 experienced regression
I/II	2010-present	DEC205 fused with HIV gag p24 + TLR3 agonist	Human immunodeficiency virus	In progress