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New generation of dendritic cell vaccines

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Dendritic cells (DC) play a pivotal role in the induction and regulation of immune responses, including the induction of cytotoxic T lymphocytes (CTL) responses. These are essential for the eradication of cancers and pathogens including HIV and malaria, for which there are currently no effective vaccines. New developments in our understanding of DC biology have identified the key DC subset responsible for CTL induction, which is now an attractive candidate to target for vaccination. These DC are characterized by expression of novel markers Clec9A and XCR1, and a specialized capacity to cross-present antigen (Ag) from tumors and pathogens that do not directly infect DC. New generation DC vaccines that specifically target the cross-presenting DC in vivo have already demonstrated potential in preclinical animal models but the challenge remains to translate these findings into clinically efficacous vaccines in man. This has been greatly facilitated by the recent identification of the equivalent Clec9A$^+$XCR1$^+$ cross-presenting DC in human lymphoid tissues and peripheral tissues that are key sites for vaccination administration. These findings combined with further studies on DC subset biology have important implications for the design of new CTL-mediated vaccines.

Keywords: dendritic cells, vaccines, targeting, tumor immunity, cytotoxic T cells

Abbreviations: Ag, antigen; CTL, cytotoxic T lymphocytes; DC, dendritic cells; cDC, conventional DC; pDC, plasmacytoid DC; mAb, monoclonal antibodies

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DC Vaccines for the Induction of CTL against Pathogens and Cancers

The success of currently available vaccines is reliant on their ability to induce serum neutralizing antibodies. However, for the development of prophylactic and therapeutic vaccines against cancer and pathogens including HIV, malaria and tuberculosis there is now a large body of evidence to suggest that the induction of cytotoxic T cell (CTL) responses are important to provide protection and control established disease. Despite intensive efforts to develop vaccines designed to induce CTL responses, there are currently no effective vaccines for these diseases. Dendritic cells (DC) are the key antigen-presenting cells responsible for the initiation of CTL-mediated immune responses against cancers, intracellular pathogens and viruses. The existence of multiple DC subsets with specialized functions is now apparent in mice but translating this to humans has been a major challenge. Several recent studies have provided new insights into the DC network in human tissues. These findings have significant implications for the design of CTL-mediated vaccines.

The Complex Network of DC: Multiple Subsets with Specialized Functions

The DC network is comprised of multiple subsets that differ in their ontology, location, phenotype and specialized function. The first division, evident in both mouse and man, occurs between plasmacytoid DC (pDC) and myeloid DC, the latter also referred to as conventional DC (cDC). PDC produce large amounts of type I IFN[1,2] and act as a first line of defense against viral pathogens, though their role in the priming T cell responses remains controversial.[3] By contrast, cDC are considered the "professional" antigen

(Ag) presenting cells critical for the activation of naïve T cells.[4,5] The cDC are further divided into "lymphoid-resident" DC and "migratory DC." The lymphoid-resident DC arrive in lymphoid organs as blood-borne precursors that develop into immature DC where they monitor the blood, lymphatics or other DC for pathogens.[5-7] In the mouse, lymphoid-resident DC are further segregated into $CD8^+$ DC and $CD8^-$ DC based on their expression of the CD8α chain.[5] The migratory DC do not develop in the lymphoid organs, but in the peripheral sites that they then monitor and sample for Ag. In the steady-state, and at an increased rate upon activation in response to pathogens or host intrinsic signals of damage, migratory DC travel to lymphoid tissues.[8] During this process they upregulate their co-stimulatory molecules and proceed to directly present their Ag to T cells[9] or share the captured Ag with lymphoid-resident DC.[6] There are multiple subsets of migratory DC depending on the location they survey.[10,11] Significant functional specializations are seen between the $CD103^-CD11b^+$ (referred to as $CD11b^+$ DC) and $CD103^+CD11b^{lo}$DC (referred to as $CD103^+$DC) and the Langerhans' cells ($CD207^+CD11b^+CD103^-$).[4] Lastly, a separate DC population, termed "inflammatory DC," originates from monocytes and develops rapidly in response to inflammation or infection. These DC probably most closely resemble the monocyte-derived DC generated in vitro in the presence of GM-CSF/IL-4.[12-14]

Defining Cross-Presenting DC and Their Role in CTL-Mediated Immunity

Although by definition all cDC are capable of processing and presenting Ag and priming naïve T cell responses, only a small subset of migratory and lymphoid-resident cDC specialize in "cross-presentation," that is the ability to present exogenous Ag in the context of MHC class I. Typically only endogenous Ag is presented in the context of MHC class I but cross-presenting DC sample Ag from other cells, circumventing the need to be directly infected by pathogens to acquire their Ag to prime CTL. In the mouse, the lymphoid-resident $CD8^+$ DC and migratory $CD103^+$ DC are the main cross-presenting DC and are crucial for the induction of CTL responses against cancers, viruses and other pathogenic infections.[15-18] Indeed, there is strong evidence that these two DC subsets are closely related. Both $CD8^+$ DC and $CD103^+$ DC have a similar transcriptional signature,[19] require BatF3,[15] Id2[20] and IRF8[18,20] for development and arise from a common precursor.[20] Initially, $CD8^+$ DC and $CD103^+$ DC were thought to be entirely dependent on Batf3 for development as exemplified by their absence in Batf3 deficient mice, which retained all other DC subsets.[15,18,21] More recent data suggests that Batf and Batf2 can compensate for Batf3.[22] Despite this, $CD8^+$ DC and $CD103^+$ DC are often referred to as Batf3-dependent DC. Delivering Ag and adjuvant directly to these DC is an attractive strategy for the induction of CTL and is, hence, being pursued in preclinical models.[10] Since human DC do not express CD8α, and CD103 is broadly expressed, translating the biology of mouse DC to human DC has been problematic. The discovery of several novel molecules exclusively expressed by these DC has permitted more refined phenotyping and functional insights and, importantly as discussed below, the identification of the human equivalents.

Bridging the Gap between Mouse and Human DC: Identification of Conserved Markers

In human, cDC have been classically defined as blood-lineage-marker negative, MHC class II^+ and $CD11c^+$ and are subdivided into CD1c (BDCA-1)$^+$ and CD141 (BDCA-3)$^+$ DC. There is now convincing evidence from a number of groups using genomics, phenotypic and functional approaches that $CD141^+$ DC in blood and lymphoid tissues are the human equivalents of the mouse lymphoid-resident $CD8^+$ DC.[23-26] Like their mouse counterpart, $CD141^+$ DC are efficient at cross-presentation, express TLR3 and respond to TLR3 ligation by producing IFN-λ.[27] However, CD141 is not an ideal defining marker since it is widely expressed on human cells and entirely absent from all mouse DC. Thus, the requirement for conserved markers between species has continued and was only fulfilled with the identification of the C-type-lectin, Clec9A, the chemokine receptor, XCR1 and the nectin-like protein, CADM1 (Necl2). Clec9A is a receptor for dead cells and a regulator of cross-priming,[28-30] while XCR1 and CADM1 play a role in $CD8^+$ T cell stimulation.[31,32] All three molecules are expressed on mouse $CD8^+$ DC and the human equivalent $CD141^+$ DC. Clec9A and XCR1 are particularly important as they provide the means to identify the human equivalent of the murine migratory $CD103^+$ DC. In the mouse, only the $CD8^+$ DC and $CD103^+$ DC express XCR1[19,33] and both DC subsets express Clec9A.[34-37] Importantly, human $CD141^+$ DC expressing Clec9A and/or XCR1 have now been identified in lymphoid and non-lymphoid tissues, including skin, lung and gut.[25,26,36,38,39] This suggests that the $Clec9A^+XCR1^+CD141^+$DC in peripheral tissues are the human equivalent of the mouse $Clec9A^+XCR1^+CD103^+$ DC, which survey the periphery and traffic to the lymphoid organs. Given the high degree of conservation in tissue localization and genomic, phenotypic and functional similarities, a unified identity for the cross-presenting DC across multiple tissue subtypes and species is now achievable. Since Clec9A and XCR1, in conjunction, are the most specific defining markers across tissues and species, we hereafter refer to this population of cross-presenting DC as $Clec9A^+XCR1^+$ DC.

Targeting Cross-Presenting DC for Immunotherapy

CTL are stimulated by activated DC that have processed and are presenting Ag in the context of MHC class I in the lymph nodes. Therapeutic vaccines utilizing peptides, recombinant proteins, viral vectors, tumor cells or lysates are "non-targeted" and rely on these agents being captured by local DC and transported to the draining lymph node for presentation to T cells. In an effort to enhance the amount of tumor Ag presented by DC, an alternative approach involved differentiating DC from monocytes in vitro, loading these with Ag and adjuvants and injecting these into patients as therapeutic vaccines. Unfortunately, these therapeutic

DC-based cancer vaccines are expensive, labor-intensive, require customization for each patient and ultimately have been of limited clinical benefit.[40,41] A more efficient vaccine strategy is to deliver the Ag directly to DC in vivo. This has been achieved by immunizing with monoclonal antibodies (mAb) that recognize cell surface receptors expressed on DC and carry antigenic cargo. Targeting Ag to the DC subsets that are ideally equipped for cross-presentation and priming of CTL responses would inherently seem advantageous. In the mouse, it is the Clec9A$^+$XCR1$^+$ DC that play a key role in the induction of CTL and since their counterpart is conserved in humans, delivering Ag to this DC subset via mAb that recognize Clec9A or XCR1 is an attractive vaccine strategy. This is now a viable option, first due to the development of anti-Clec9A and anti-XCR1 mAb that can deliver Ag specifically to Clec9A$^+$XCR1$^+$ DC in vivo,[33,42] mediating cross-presentation and CTL induction.[34,37,43,44] Second, it is now clear that these cells are located in tissues such as skin[38] and lung[36] where vaccine administration (i.e., intradermal or intranasal) is not only practical but has been clinically demonstrated to be more effective with lower doses of Ag compared with the standard injection routes (i.e., intramuscular, subcutaneous).[45,46]

Targeting Cross-Presenting DC in the Mouse: What have We Learnt?

In the mouse, extensive work has been published on targeting Ag to DEC-205, a multi-lectin receptor expressed at high levels of CD8$^+$ DC (reviewed elsewhere[10]). This body of work has made two clear observations. First, effective priming of CTL requires the delivery of Ag in the presence of DC activation/maturation signals[47,48] and second, targeting the subset of DC that cross-present results in superior CD8 T cell responses.[49,50] In the mouse, many other receptors have been exploited for the delivery of Ag and these studies have made a number of other salient points.[51] For example, it is logical to assume that the best receptor for Ag-delivery should only be expressed on DC; indeed promiscuous expression by other APC may prove detrimental. However, targeting Ag to DEC-205,[48] CD36,[52] CD11c[53] and Clec12A,[44,54] all of which are expressed on multiple cell types, induced strong CD8 T cell responses. Importantly though, while the broad expression patterns of these receptors did not prevent the induction of CTL, it was the DC and not the other cells that were responsible for the priming of T cell immunity.[54-56] Our own data also warns that not all receptors expressed by CD8$^+$ DC will automatically be good vaccine targets. In this vein, though DEC-205, Clec9A and Langerin were comparable at promoting CD8 T cell responses,[57] delivering Ag to Clec12A, which is also expressed on CD8$^+$ DC (as well as other DC subsets and non-DC) was significantly less effective at promoting cross-presentation.[44] The capacity of the individual receptor to promote cross-priming is critical when considering it a vaccine target. We and others have already confirmed that targeting Ag to Clec9A is extremely effective at promoting the priming of CTL[37,44] and generating protective anti-tumor responses.[37] The question remaining to be answered is whether XCR1, the other receptor exclusively expressed on the cross-priming DC can be used to induce CTL. Since an anti-XCR1 mAb has recently been generated,[33,42] it will be possible to compare Ag delivery to these receptors and determine which is most effective at promoting the induction of CTL. The last point that needs consideration is whether the mAb itself may affect immune outcome. In the case of Clec9A, one mAb elicits humoral responses in the absence of adjuvants, while another requires adjuvant.[34,43,44,51] Since neither mAb appears to directly activate DC, it is difficult to reconcile these differences and this is the subject of a current collaborative study. However, in terms of inducing CTL responses, both of these mAb to Clec9A require co-administered adjuvants for efficacy,[37,44] clearly indicating this will be the optimal targeting protocol for future clinical trials.

What is the Role of Other DC-Subsets in Anti-Tumor and Anti-Viral Immunity?

In the mouse, the crucial role for Clec9A$^+$XCR1$^+$ DC in the induction of anti-tumoral and anti-viral immune responses has been established,[15,18] though the individual contributions of migratory vs. lymphoid-resident Clec9A$^+$XCR1$^+$ DC remains unknown. Even less is known about the contribution of lymphoid-resident CD8$^-$ DC and CD11b$^+$ migratory DC, to anti-tumor and anti-viral immunity. There is some evidence that at least some subtypes of these DC are specialized at inducing CD4$^+$ T cell responses.[9,58-60] This may be an important consideration in vaccine design for maximizing CD4 T helper and humoral immune responses. Another important function of migratory CD11b$^+$ cDC may be to transfer Ag to lymphoid-resident Clec9A$^+$XCR1$^+$ DC[6] but the significance of this process in CTL-mediated immunity is yet to be elucidated. The absence of a unique transcription factor defining the CD11b$^+$ cDC subsets makes it difficult to discern their function in vivo. There are currently no definitive markers that clearly align the CD11b$^+$ cDC subsets across different tissues and species.

In the mouse, under inflammatory conditions, monocytes can also acquire DC-like features[12] and effectively cross-present targeted Ag.[61] The equivalent of these in vivo-induced monocyte-derived DC remain to be identified in humans. Whether these monocyte-derived DC can be exploited for Ag-delivery and induction of CTL remains to be determined. Ultimately, it is the identification of definitive new markers that will facilitate the translation of mouse DC-biology to human DC-biology and identify the role these DC subsets play in cross-priming.

Which Adjuvant Will Be Most Effective?

One of the most crucial lessons of DC clinical trials has been the requirement for DC activation in order to generate CTL responses.[40,41] This is also a pre-requisite for the induction of CTL in mice when Ag is delivered via mAb in vivo.[10] Indeed, delivering Ag and adjuvant simultaneously to DC enhances immunogenicity.[62-66] In terms of DC immunotherapy this infers that the adjuvant must be delivered to the same DC subset being targeted with Ag. TLR ligands such as poly I:C (TLR3), MPL (TLR4) and CpG (TLR9)

have all demonstrated efficacy as potent vaccine adjuvants in mice and non-human primates and are now being trialed in humans. Poly I:C is particularly effective as an adjuvant for T cell immunization that is well-tolerated in humans and elicits a Type I IFN signature that mimics live virus infection.[67] The study of mouse and human DC have alerted to one important interspecies difference, namely while all mouse Clec9A$^+$XCR1$^+$ DC express and TLR3, 4, 9[68] and respond to these TLR-ligands, human Clec9A$^+$XCR1$^+$ DC only express TLR3.[25,69] In light of this information, poly I:C or its stabilized analog (LC:IC) is currently one of the most attractive adjuvants to incorporate into vaccines aimed at targeting human Clec9A$^+$XCR1$^+$ DC.

Concluding Remarks and Future Directions

The discovery of the Clec9A$^+$XCR1$^+$ cross-presenting DC in mouse and man has provided new insights into the induction of CTL responses against viruses and tumors and paves the way for rational vaccine designs. Key questions that remain to be answered are: which molecule is the best receptor for Ag delivery, which adjuvant is most effective and ultimately, will targeting this DC subset alone be sufficient to generate protective immunity? In this regard further characterization of less well defined DC subsets and their role in viral and tumor immunity is essential. Though Clec9A$^+$XCR1$^+$ DC have been analyzed in their steady-state, quantitative and qualitative changes in response to infection and cancer may affect the ability to therapeutically target these DC in vivo and will need to be carefully evaluated in human disease settings. For cancer, in vivo targeting of DC will likely be most effective in combination with other agents that overcome the immunosuppressive environment such as targeting CTLA4 or PD1, or enhancing "immunogenic" tumor cell death with chemotherapeutic agents.[70] For priming viral immune responses, DC targeting may be more effective when used in combination with other vaccines as a prime-boost strategy, as recently shown for the induction of HIV responses using DEC-205 targeting in non-human primates.[71] Finally, translating promising findings from preclinical animal models into effective human vaccines remains a major challenge. The profound advantages of targeting DEC-205 in mice were more modest in non-human primate studies.[71,72] However, the first proof-of-concept clinical trials using DEC-205 to target DC in vivo in healthy volunteers are underway[72] and the results are eagerly anticipated.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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