Review

Targeting C‐type lectin receptors: a high‐carbohydrate diet for dendritic cells to improve cancer vaccines

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Abstract

There is a growing understanding of why certain patients do or do not respond to checkpoint inhibition therapy. This opens new opportunities to reconsider and redevelop vaccine strategies to prime an anticancer immune response. Combination of such vaccines with checkpoint inhibitors will both provide the fuel and release the brake for an efficient anticancer response. Here, we discuss vaccine strategies that use C‐type lectin receptor (CLR) targeting of APCs, such as dendritic cells and macrophages. APCs are a necessity for the priming of antigen‐specific cytotoxic and helper T cells. Because CLRs are natural carbohydrate‐recognition receptors highly expressed by multiple subsets of APCs and involved in uptake and processing of Ags for presentation, these receptors seem particularly interesting for targeting purposes.

Abbreviations

cDC
    conventional dendritic cell

CLR
    C‐type lectin receptor

DC
    dendritic cell

DCIR
    dendritic cell immunoreceptor

DC‐SIGN
    dendritic cell–specific intercellular adhesion molecule‐3‐grabbing nonintegrin

dDC
    dermal dendritic cell

Flt3L
    fms‐like tyrosine kinase‐3 ligand

GLA
    glucopyranosyl lipid A

hCG‐β
    human chorionic gonadotropin β‐chain

HEL
    hen egg lysozyme

LC
    Langerhans cell

Le
    Lewis

LN
    lymph node
MGL
    macrophage galactose - type lectin
moDC
    monocyte - derived dendritic cell
MR
    mannose receptor
pDC
    plasmacytoid dendritic cell
PD - L1
    programmed death - ligand 1
PLGA
    poly(lactic - co - glycolic acid)
poly - IC
    polyinosinic–polycytidylic acid
RECIST
    response evaluation criteria in solid tumors
scFv
    single - chain Fv Abs
Siglec
    sialic acid–binding Ig - type lectin

Introduction

Clinical studies have demonstrated the potency of checkpoint inhibitors to unleash endogenous T cell responses against a variety of tumor types [1, 2]. Tumor types with a high mutational load, such as melanoma and non–small cell lung cancer, can be infiltrated by naturally primed cytotoxic T cells that, unfortunately, tend to be subsequently suppressed by soluble - and contact - dependent factors in the tumor microenvironment. Upon reactivation with checkpoint inhibitors, these effector T cells can mediate regression that, in many cases, has a proven, durable effect. Unfortunately, most patients do not develop sufficient immune response after treatment with checkpoint inhibitors to achieve a clinical response. Absence of a clear T cell infiltrate has been linked to an apparent inability to respond to an immune checkpoint blockade [3]. A recent study has suggested that this might be due to a lack of recruited and properly activated DCs, rather than a lack of nonsynonymous mutations providing immunogenic neoepitopes [4]. This observation points to the pivotal role of APCs in kick - starting and maintaining a powerful antitumor immune response. As such, patients with "cold" tumors (i.e., those lacking an effector T cell infiltrate) could benefit from therapeutic vaccination with neo - or shared tumor Ags to induce, recruit, and maintain an anticancer cytotoxic T cell response, which might then render those tumors responsive to immune checkpoint blockade.

Therapeutic vaccination against cancer will have to rely on efficient Ag loading and activation of DCs to prime powerful antitumor T cell immunity, even in the face of the various immune - suppressive barriers put up by the tumor. This review will focus on the advantages of using the unique in vivo Ag - capturing, - processing, and - presenting abilities of APCs, specifically DCs and macrophages, by targeting vaccine formulations to CLRs expressed on their cell surface. An increased understanding of the expression and functionality of these receptors on specific APC subsets in various tissue compartments now allows for the rational design of optimized in vivo APC - targeted tumor vaccines. We will discuss the various considerations that should be taken into account to arrive at viable clinical applications that may prove a valuable addition to the ever - growing arsenal of immunotherapies in the battle against cancer.

DC - BASED TUMOR VACCINES: FROM IN VITRO TO IN VIVO

Vaccination with ex vivo cultured moDCs or CD34^{+} progenitor - derived DCs has been around for decades. Although clinical benefit has been reported, such DC - based vaccines have generally not been able to deliver the anticipated clinical effects. Part of this failure could be due to a lack of consistency in the vaccine preparations, i.e.,

the choice of immature or mature DCs, the choice of DC subsets, the culture methods, the administration route, and the Ag - loading protocols [5]. In general, administration of mature ex vivo - cultured DCs seems to result in better migration and improved clinical response compared with the response induced by immature DCs. In addition to autologous moDCs or CD34^{+} progenitor - derived DCs, more recent work has focused on the generation of other, more - specialized DC subsets, such as umbilical cord blood–derived interstitial - and LC - like DCs [6, 7], the development of DCs from allogeneic cell lines, or the administration of low numbers of isolated, and in vitro–loaded, autologous blood DC subsets, such as CD1c/BDCA1^{+} DCs or pDCs [8–10].

Given their potentially different functions, in vivo targeting of (specific) tissue DC subsets through specific surface receptors might be more efficient (reviewed in [11, 12]). An advantage of this approach over ex vivo administration is that it is less laborious and the targeted DCs will be present in their natural environment, with naturally efficient homing and migration capacities (e.g., to the LNs, which proved a major hurdle in earlier moDC - based vaccines). Thus far, in vivo APC targeting in aid of antitumor immunity has mostly been tested in preclinical models. Among others, successful examples were based on targeting DCs through CLRs that efficiently mediate uptake, processing, and presentation of Ags to T cells.

APC SUBSETS: DCs AND MACROPHAGES

When contemplating what APC subset(s) to target in vivo for tumor vaccination, it is important to consider targeting receptors and functional differences between APC subsets. This has long been complicated by a general lack of specific surface markers and unclear relationships between APC subsets in mice and their counterparts in humans. DCs have classically been identified by the analysis of a limited number of specific surface markers. However, because surface markers are very tissue specific and species restricted, this approach precluded a simplified and unified classification of DCs among different tissues and species. Recent technical developments have allowed the identification of different types of DCs that are not only based on their in vitro dependency on growth and transcription factors but also on signature transcriptional expression profiles that are conserved among different tissues and species. These studies have also provided greater insight into the phenotypic and functional differences between macrophages and DCs.

Three main types of DCs exist in mice and man

In both mouse and man, roughly 3 main types of DCs can be distinguished: pDCs, cDCs, and moDCs. The generation of pDCs is dependent on the transcription factor E2 - 2 in both mice and men [13, 14]. pDCs and cDCs have a short $t_{1/2}$ of 3–7 d, and peripheral and/or lymphoid tissues continuously receive cDC and pDC precursors from the blood [15, 16]. The generation of pDC and cDC is dependent on the growth factor Flt3L [17–19]. Mouse cDC from lymphoid and peripheral tissues are characterized by high CD11c and MHC class II expression, but additional markers are necessary to exclude moDCs and macrophages, especially in peripheral tissues [20, 21]. cDCs can be subdivided into 2 subsets; the differentiation of which depends on specific transcription factors, and for which a new nomenclature was recently proposed [22]. Mouse cDC1s were originally defined by the expression of CD8 α in lymphoid organs and CD103 in the periphery, whereas, in human blood, they were characterized as CD141/BDCA3^{high} cells, but this subset is more specifically characterized by the expression of XCR1 and the C - type lectin CLEC9a/DNGR1 [23–28]. The transcription factors IRF8 and batf3 guide the differentiation of cDC1s in mice and in humans [29–35]. Mouse cDC2s express CD11b, and are specifically characterized by SIRP α expression in both mice and in humans [34, 36]. The separation of cDC1 and cDC2 subset lineages appears to occur in the bone marrow because already committed precursors can be identified there, as well as in the blood, of both mice and man [37, 38].

Next to pDCs and the 2 cDC subsets, a moDC subset can be identified that is dependent on CSF1, but not on Flt3L, and is difficult to discriminate from macrophages. Because macrophages themselves show heterogeneity in origin and marker expression, depending on the tissue, there is a large variation in nomenclature for these different monocyte - derived cell types. Recent studies have shown that, in mice, most tissue - resident F4/80[bright] macrophages are derived from embryonic precursors that proliferate locally in the tissues under the control of CSF1 or IL - 34 (reviewed in [39]). A limited number of tissues, such as the intestine and skin, contain macrophages that continuously differentiate from blood - derived monocytes [40–42].

In mice, the expression profiles of cDCs and tissue resident macrophages have been extensively analyzed and specific gene - expression signatures have been defined for both cell types [20, 21]. In general, the transcriptional signature of cDCs includes CCR7, Flt3, Zbtb46, c - Kit, and Btla, whereas macrophages can be identified by the expression of CD64, MerTK, TLR4, G - CSFR, and CD14. The inclusion of these signature markers has helped in the

characterization of cDCs and resident macrophages. However, during inflammation, monocytes can differentiate into both moDCs and macrophages, and the distinction between these 2 subsets is just beginning to be defined [43–47]. A recent study indicated that blood Ly6C+ monocytes contain 2 different precursors that differentiate into either GM‑CSF–dependent PD‑L2+CD209a+ moDCs or into PD‑L2−CD209a− macrophages that express iNOS [48]. That was the first study that better defined moDCs and monocyte‑derived macrophages, and additional studies are required for better definitions for these cell types.

Functions of DC subsets and macrophages

Although DCs and macrophages both take up Ags and have a very similar transcriptional and phenotypic profile, the current view is that different types of DCs and macrophages have specific functions, but that redundancy may also exist between subsets for those functions. pDCs are characterized by the high and rapid release of type I IFN after viral infection, and their main role is the stimulation of antiviral, innate and T cell‑mediated, immune responses [49–51]. cDCs have been shown to be the most important cell type to prime naive T cells, and for migratory subsets this is correlated with their capacity to migrate from peripheral to lymphoid tissues and the expression of CCR7 [21, 52]. In mouse models, cDC1s have the best capacity to cross‑present cell‑associated Ags and are essential for the activation of Th1 and CD8 T cell response against several pathogens [29, 53, 54]. With regard to the induction of antitumor T cell responses, cDC1s are also essential for the cross‑presentation of tumor Ags and the activation of tumor Ag‑specific CD8 T cells, and expansion and activation of cDC1c enhances immune responses stimulated by checkpoint inhibitors [55–57]. Alternatively, cDC2s have been shown to stimulate Th2 and Th17 CD4 T cell responses and to stimulate B cell responses [58–62]. These observations have led to a model in which cDC1s are necessary for immune response against intracellular pathogens, whereas cDC2s stimulate immune response against extracellular bacteria, fungi, parasites, and allergens [63]. In addition, recent studies have indicated that, although during the initial immune response, CD4 and CD8 T cells are activated by different DC types, later in the response, cDC1s provide a platform for both CD8 and CD4 T cell activation, which enables CD4 T cell help and long‑term memory CD8 T cell responses [64, 65].

Although moDCs have shown strong Ag (cross)‑presentation and T cell activation capacity in vitro [66, 67], it seems that the cross‑presentation machinery is different between cDCs and moDCs [66, 68–70]. Furthermore, the extent to which moDCs can travel to LNs and contribute to the induction of naive T cell responses in vivo is still being debated.

In man, this division of tasks among DC subsets for determining the quality of the generated immune response is far less clear. Cross‑priming abilities have been observed for virtually all cDC subsets studied in human LNs and skin [71]. In fact, transcriptional profiles of the various human cDC subsets in peripheral tissues appear to be determined by the tissue microenvironment in which they reside [72], whereas the identity of DCs in the lymphoid organs is dictated by ontogeny [73]. The prevailing, local cytokine balance in the periphery in large part defines the Th‑skewing abilities of migratory cDC subsets, resulting in preferential Th1, Th2, Th17, or T_{reg} induction (reviewed in [74]). Thus, for DC‑targeted tumor vaccines, the codelivered immune adjuvants may prove vital in this respect.

Whereas the main function of DCs is to stimulate naive T cell responses, macrophages exert a large variety of local activities in peripheral tissues and do not travel to LNs to stimulate T cells but may be involved in the maintenance of resident memory T cells [75–77]. One specific type of macrophage, which is situated in the lymphoid tissues and is characterized by high expression of the lectin Siglec‑1/sialoadhesin/CD169, may have a facilitating role in the activation of adaptive immune responses. In LNs, CD169+ macrophages are situated adjacent to the subcapsular sinus, and in the spleen next to the marginal sinus, from which they pick up Ags [78, 79]. These macrophages present Ags in intact form to B cells and stimulate B cell responses but also transfer Ags to cDC1s for activation of CD8 T cell responses, and they may also stimulate CD8 T cell response to some epitopes themselves [80–83]. The exact role of this specialized macrophage subset and its crosstalk with migratory and LN‑resident DC subsets in human lymphoid tissues remains to be established.

DCs in the skin

The skin comprises an extensive network of specialized DC subsets and, remarkably, at any given time, contains more T cells than peripheral blood does [84, 85]. Combined with a dense and finely branched lymph vasculature, representing a fast‑track delivery system to regional LNs, these traits make the skin an ideal portal for the (targeted) delivery of tumor vaccines [86]. The skin can be separated into the epidermis and dermis, which each contain specific myeloid cell types. LCs were originally considered the most important DC subtype of the skin, but

several studies have shown that the underlying dermis also contains cDCs that migrate to the LNs and stimulate T cell responses (reviewed in [87–89]). Recent ontogeny studies demonstrated that LCs depend on the IL‑34‑CSFR1 signaling for their maintenance, and this led to a proposal to classify LCs as macrophages [90–93]. Under steady‑state conditions, LCs stem from primitive yolk sac–derived precursors that have seeded the epidermis and then proliferated locally [22, 94]. Under inflammatory conditions, LCs can be derived from recruited monocytes and DC precursors [95, 96]. Moreover, gene expression studies showed that human LCs, in particular, show high expression of cross‑presentation machinery and overlap considerably with cDC expression profiles, which, together with their capacity to migrate to LNs and their capacity to stimulate T cell responses in vitro, suggests that LCs form an intermediate between macrophages and DCs [97, 98]. LCs can be identified by their high expression of EpCAM, Langerin, and CD1a.

In both mouse and human skin, dDCs can be separated into cDC1 and cDC2 subsets, roughly and operationally defined based on XCR1 and SIRP α in mouse and CD141 and CD1c/SIRP α in man [99], although CD141 can be de novo up‑regulated on cDC2s under the influence of IL‑10 [100]. In mice, cDC1s express Langerin, similar to LCs [101], which can be used for targeting [102–104]. Human CD1a$^+$ dDCs that had migrated to tumor‑draining LNs were shown to express Langerin intracellularly [105, 106]. In both mouse and man, an additional moDC subset and macrophages can be identified in the dermis. In mice, macrophages are characterized by CD64 and MerTK expression and the absence of Ly6C, whereas moDCs are MerTK$^-$, CCR2$^+$, and Ly6C$^+$ and can migrate to LNs albeit in low numbers [107]. Similarly, in human skin, moDCs and macrophages can be identified: whereas both express markers such as CD14, CD11b, SIRP α, CD64, CD163, and CD209, macrophages can be recognized by their autofluorescence and Lyve‑1 and FXIIIa expression [108].

Based on differential CD1a and CD14 expression in man, at least 4 CD11c^{high} interstitial dDC subsets can be discerned, including CD1a$^+$, CD14$^+$, and double‑negative and double‑positive dDCs [109, 110]. The frequency distribution among these migratory subsets, and thereby the eventual T cell activation outcome, depends on the activating vs. regulatory/tolerizing cytokine balance in the skin microenvironment [109]. Under the influence of suppressive IL‑10, migration of CD14$^+$ dDC/macrophage‑like cells prevails, resulting in abortive T cell priming and T$_{reg}$ induction and expansion. Under proinflammatory conditions (e.g., at high interstitial levels of GM‑CSF and IL‑4), migration of CD1a$^+$ LC and dDC subsets is dominant, leading to Th1 and CD8 cytotoxic T cell induction and expansion [109]. Thus, the frequency distribution of migratory dDC subsets from human skin determines T cell activation vs. tolerance induction [74], which is an important consideration in the design of DC‑targeted tumor vaccines. Of note, a genome‑wide transcriptional profiling analysis of freshly isolated human CD1a$^+$ dDCs vs. LCs showed dDCs to express a far wider range of adhesion and costimulatory molecules, chemokines, and cytokines (and at higher levels), pointing to a putatively superior migratory and T cell stimulatory ability over LCs [111]. Indeed, a comparative ex vivo study of the ability of these human cDC subsets to prime CD8 effector T cells against a MART‑1–derived HLA‑A2–restricted epitope is in keeping with these transcriptional analyses and demonstrated a superior capacity of migrated dDCs to prime cytotoxic CD8 T cells, both in numbers and functional avidity [112].

DCs in skin‑draining LNs

As vaccines delivered through the skin can drain either directly to LNs or be carried there eventually by migratory APC subsets, it is important to characterize the DC subsets residing in the skin‑draining LNs. From comparative studies with human skin explants, both skin‑derived migratory subsets (CD1a$^+$ LCs and dDCs) can be identified in human skin‑draining LNs [105, 106]. These CD1a$^+$ migratory subsets express relatively high levels of costimulatory molecules, CD83, and CCR7. Administration of GM‑CSF (i.d.) leads to further up‑regulation of their costimulatory machinery and CD83 and to increased numbers of these DCs in the paracortical LN areas [113]. Frequencies of these mature CD1a$^+$ DCs in melanoma‑draining sentinel LNs correlated significantly with melanoma‑specific CD8 effector T cells, indicative of the validity of this DC subset for tumor vaccine targeting [114].

In addition to migratory LN subsets, LN‑resident subsets were identified with a numeric predominance of a CD11c^{high}CD14$^-$ cDC subset. These LN‑resident cDCs do not express CD1a but do express CD83 and costimulatory molecules on their surface, albeit at considerably lower levels than the CD1a$^+$ migratory cDC subsets [105]. Frequencies of this DC subset were up‑regulated in melanoma‑draining sentinel LNs upon administration of the TLR9 agonist CPG7909 [115]. Expression of the CLR BDCA3/CD141 and CLEC9a on at least part of these cDCs suggested that the BDCA3$^+$ cDC1 subset in peripheral blood might be their direct precursor. Indeed, upon CPG7909 administration, recruitment of these LN‑resident cDCs from cDC1 in the peripheral blood was

upon CPG7909 administration, recruitment of these LN‑resident cDCs from cDC1 in the peripheral blood was strongly suggested by a significant reverse correlation of the frequencies of these subsets in the LN vs. the blood [116]. In addition to that subset, classic cDC1s with high levels of CD141 and CLEC9a are present in skin‑draining LNs [106]; however, the possibility that this may be due to blood contamination cannot be excluded. Moreover, an apparent equivalent capacity for cross‑priming for all cDC subsets in human LNs calls the relative significance of this small number of cDC1s into question [117].

Finally, a CD14$^+$ LN‑resident DC subset can also be discerned. Although at least part of this subset most likely represents monocytes or macrophages, low‑level CD83 expression on a subpopulation seems to suggest a semimature DC phenotype. These DCs do not derive from migratory CD14$^+$ dDC/macrophage‑like cells because they are immature and do not express CD83 [105]. Moreover, they are clearly distinct from a population of M2‑like macrophages expressing CD163 and PD‑L1, which are recruited to tumor‑containing LNs [118].

Remarkably, although migratory subsets expressed higher levels of costimulatory molecules and appeared to be in a greater state of maturation, the LN‑resident cDC subsets (both CD14$^{-−}$ and CD14$^+$) displayed a superior ability to prime IFN‑γ–expressing allogeneic T cells [105]. Their frequencies also correlated significantly with released levels of IL‑1β, IL‑6, and IL‑12p70 and cross‑presentation capacity in 24‑h, ex vivo, sentinel LN cultures [105, 116]. These observations clearly point to the validity of specifically targeting these LN‑resident cDC subsets in aid of tumor vaccination.

In conclusion, the plethora of studies in mice and man on different types of DCs in peripheral and lymphoid tissues have demonstrated their absolute requirement in the initiation of adaptive immune responses in general as well as the activation of cytotoxic T cells against cancer in particular. These studies also indicate that multiple subsets of DCs are present, which appear to exert different functions and express different CLRs. Vaccination strategies should take into consideration the expression profiles of CLRs on DCs, as well as the immune responses that these DCs will elicit.

CLR EXPRESSION ON DCs

APCs, such as DCs and macrophages, express various lectins, including CLRs. Some of these lectin receptors are unique for a specific subset of DCs or macrophages, and expression of lectins is often regulated by maturation status of the APC [119–121]. Most CLRs recognize glycosylated Ags or apoptotic cells through specific recognition of carbohydrates or structures that bind the C‑type lectin domain. Binding of ligands can lead to activating or suppressive downstream signaling events, which regulate DC maturation, migration, and cytokine production. In addition, ligand binding generally results in internalization and processing for loading on MHC class I and/or II molecules, and the innate recognition of pathogens by CLRs is often involved in the activation of adaptive immune responses. The restricted expression of certain CLRs on specific APC subsets suggests that certain APC subsets are better equipped than others for the recognition of specific pathogens, based on the expression of carbohydrates. An example is the expression of Langerin (CD207) on LCs in the epithelial layer of the skin, which has a unique carbohydrate specificity for high mannose and LeY carbohydrates and is involved in the recognition of various viruses [122–124]. In contrast, the CLR DC‑SIGN (CD209) is merely expressed on moDCs and on CD14$^+$ dDCs in dermal layers of the skin and has specificity for mannose and all Lewis type carbohydrates (Lewis A, B, X, and Y) [125]. DC‑SIGN is often coexpressed with the MR (CD206) that shares its mannose specificity [126].

Another CLR shown to have a role in mediating T cell responses is DCIR (CLEC4a), which has a broad carbohydrate specificity for mannose and fucose [127, 128]. Different from other CLRs, DCIR contains an ITIM motif and, upon triggering with Abs, inhibits the production of inflammatory cytokines. Therefore, this receptor has been associated with homeostatic control and control of inflammation [129–131]. In addition, MGL, which is mainly expressed on immature moDCs and macrophages, has regulatory properties. It binds to galactose and N‑acetylgalactosamine and has 2 homologs in mice, MGL1 and MGL2, and mouse MGL2 has a similar binding preference as human MGL has [132, 133].

Next to these CLRs with well‑defined carbohydrate specificity, DCs and macrophages are known to express other CLRs that lack characterized carbohydrate specificity or have poorly defined ligands. These C‑type lectins have also been studied for targeting purposes to improve Ag presentation to CD4 and CD8 T cells. Examples are CLEC9a/DNGR1 and CLEC12A, both expressed on CD8$^+$ DCs and pDCs, and Ab‑mediated targeting of Ags to these CLRs has resulted in efficient cross‑presentation. The most well‑known CLR studied for the induction of CD4 and CD8 T cell responses is DEC205 (CD205), expressed on cDC1s in mice and on a wide variety of leukocytes in humans. The studies on Ag targeting to DEC205 on DCs using Abs were among the first proof‑of‑concept

studies to demonstrate that efficient activation of T cell responses was achieved after in vivo Ag targeting to DCs [54, 134–136].

TARGETING OF TUMOR AGS TO CLRs ON MACROPHAGES AND DCs USING ABS AND LIGANDS

Because DCs are essential for the induction of immune responses and because these cells show specific expression of endocytic CLRs, a tumor vaccine that targets Ags to these receptors would be expected to efficiently induce immune responses. Most pioneering work in the field of Ag targeting with Abs to lectins in vivo was performed using DEC205‐specific Abs, whereas later studies used Abs directed against DCIR, CLEC9a, CLEC12a, Dectin‐1, DC‐SIGN, MR, and Langerin (**Fig. 1**) (reviewed in [137]). Although Abs have a high affinity for their target and also have a long $t_{1/2}$ in the circulation, the use of targeting strategies that incorporate natural or artificial (glycan) ligands has gained interest during the past few years because of the added advantages of easy production and low immunogenicity [138]. The spatial orientation of the ligand and the multivalency of the ligand can be adjusted to enhance affinity of carbohydrate–CLR interactions, thereby increasing targeting efficiency and promoting CLR clustering [139]. For both Abs and ligands, many methods exist through which they can be conjugated to a variety of Ag formulations and vaccine vehicles (Fig. 1). Abs or ligands can be directly (molecularly or chemically) conjugated to tumor Ag peptides, proteins, or DNA; incorporated in nanoparticles, such as PLGA particles, or liposomes; or incorporated into adenoviruses and lentiviral vectors. This variety of use actually emphasizes the broad spectrum of possibilities for DC targeting and its innumerous opportunities for efficient Ag delivery to DCs in vivo and the induction of antitumor responses. In the next section, different Ag formulations will be discussed.
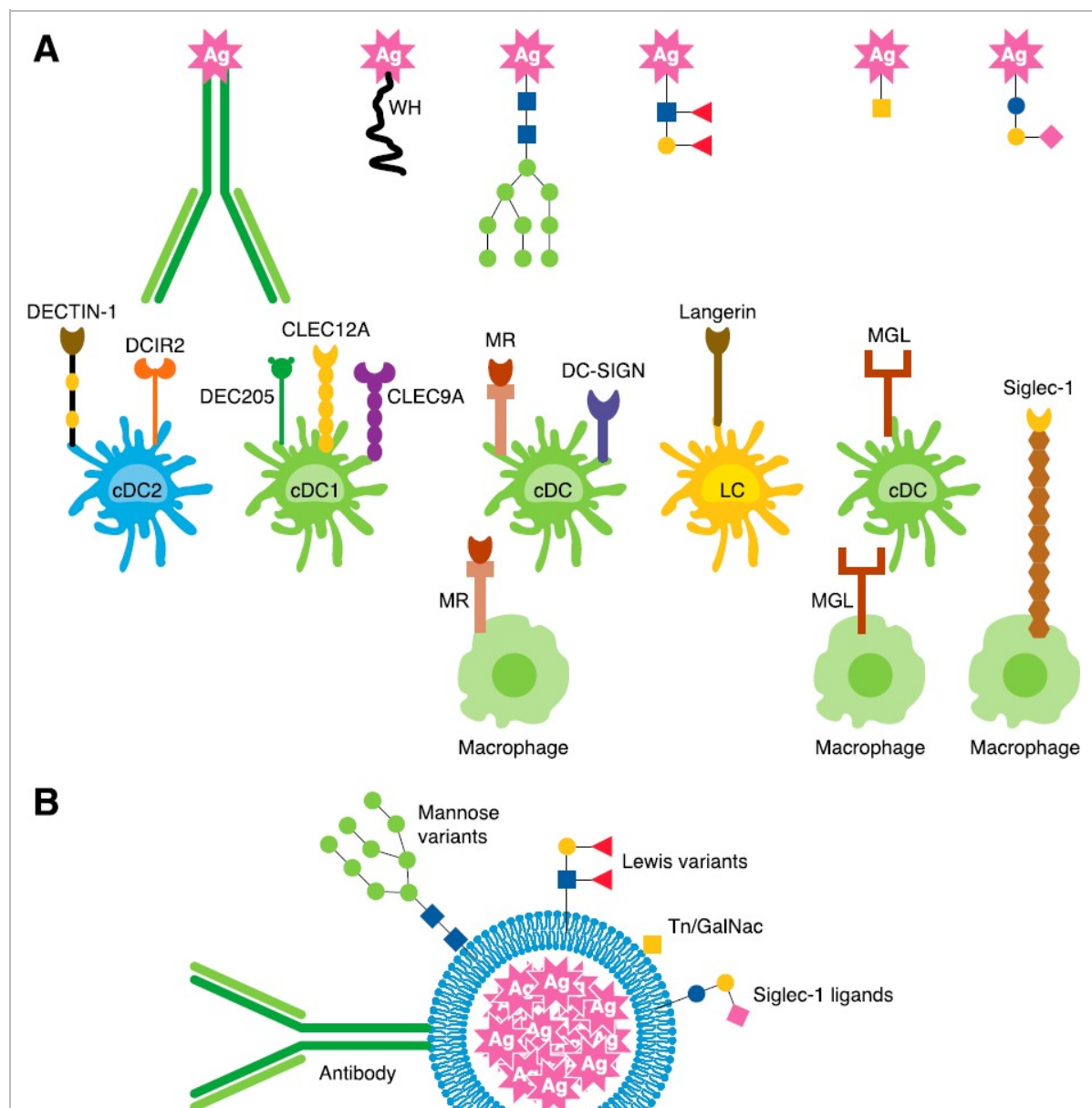
Figure 1

Subsets of DCs express different CLRs, which can be targeted by Abs or by carbohydrate ligands. (A) The expression of CLRs on cDC1s and cDC2s, LCs, dDCs, and macrophages is depicted for human and mouse subsets. All CLRs illustrated in the figure, except for MGL, have been targeted with Ab–Ag conjugates and have been shown to result in Ag presentation and T cell activation. Ags have also been targeted to CLEC9a, MR, DC‑SIGN, Langerin, MGL, and Siglec‑1 through conjugation with CLR‑specific ligands. These include WH‑peptide for CLEC9, mannosylated Ags for MR and DC‑SIGN, Lewis Ags for DC‑SIGN and Langerin, Tn‑Ag for MGL, and sialylated carbohydrates for Siglec‑1. (B) Ligands or Abs can be conjugated to Ags directly but can also be incorporated in nanoparticles, such as liposomes. DEC205 is expressed on cDC1s and LCs in mice, but in man, it is present on multiple DC subsets. For simplicity, we have only depicted DEC205 on cDC1s. DC‑SIGN is only expressed on human DCs.

Caption ⌄

Targeting to DCs with Ab against DEC205

In the first report using CLRs for DC targeting, HEL was targeted to DCs using a DEC205‑specific mAb chemically conjugated to HEL. Using adoptively transferred, HEL‑specific, TCR transgenic CD4 T cells, induction of tolerance to the Ag was shown in the absence of adjuvant, whereas the coinjection of anti‑CD40 Ab to activate DCs during the vaccination phase resulted in prolonged and increased proliferation of CD4 T cells [134]. These experiments demonstrated that the dose necessary for DC targeting was much lower (100–1000‑fold) than immunization with free Ags [134]. Compared with other immunization approaches, such as ex vivo, pulsed, splenic DC injection or free OVA protein with either CFA or anti‑CD40, DEC205 targeting with anti‑CD40 resulted in enhanced CD8 T cell activation, which, in turn, resulted in the growth inhibition of established B16–OVA melanoma tumors [135, 136]. These studies were the starting point of many others using DEC205 and other CLRs to target DCs in different settings and tumor models and led to new insights on the function of DCs. One of the first studies comparing DEC205 and DCIR2 targeting elegantly demonstrated that mouse cDC1 and cDC2 subsets have different capacities to stimulate CD8 and CD4 T cells [54].

Many subsequent studies used recombinant, scFv specific for DEC205, which was conjugated to tumor Ags, incorporated in vesicles or particles, or incorporated in DNA vaccines. The targeting of gp100 tumor Ag conjugated to DEC205‑specific scFv administered 5 d after tumor inoculation resulted in the inhibition of B16/F10 and RET tumor growth [140]. Vaccination with DNA vectors encoding a fusion protein of DEC205‑specific scFv and tumor Ags resulted in superior specific humoral and cellular immune responses, compared with the untargeted cDNA administration [141]. A similar approach using an scFv‑DEC205 DNA vaccine specific for HER2/neu ectodomain in a prophylactic, prime‑boost vaccination strategy resulted not only in specific protection of tumor outgrowth, but in addition and clinically more relevant, therapeutic treatment of established tumors combined with temporal depletion of T_{regs} also lead to improved tumor protection and survival [142]. A microbe‑based delivery system, consisting of a filamentous bacteriophage with a scFv for DEC205 resulted in tumor‑specific immune responses in the absence of any adjuvants, suggesting that this vehicle not only delivered tumor Ag to a specific DC subset but also functioned as an adjuvant [143]. Incorporation of DEC205‑specific Abs into particles and vesicles induced the activation of tumor‑specific T cell responses and regression of tumors [144–146]. These combined data, and data from other studies, indicate that DEC205‑specific Abs induce good antitumor immunity and have the potency to be translated into humans.

Anti‑CD40 and poly‑IC are commonly used in murine studies as adjuvants for lectin‑targeted vaccines. However, it is important that the adjuvants are applicable to the human system as well. GLA, a synthetic TLR4 ligand, has been tested in combination with DEC205 targeting, and it improved CD4 and CD8 T cell and Ab responses [147]. In addition, in a human vaccine trial, GLA was found effective and safe [148]. The codelivery of adjuvants to a specific DC subset might reduce side effects from the activation of other cells and enhance the specific activation of the targeted DCs. To study this, anti‑DEC205 Ab was coupled both to Ag and the TLR9 adjuvant CpG and tested in a murine model. Although this resulted in stronger immune responses, the

adjuvant CpG and tested in a murine model. Although this resulted in stronger immune responses, the combination of Ags and CpG targeted this complex also to DEC205⁻ cells, so an important lesson to be drawn from that study is that specificity should be preserved when altering the targeting moiety [149]. Nevertheless, targeting adjuvant (poly dA:dT) linked to anti‑DEC205 or anti‑Langerin improved the CD4 and CD8 T cell responses compared with untargeted adjuvant administration [150]. These preliminary data are promising, and future DC‑targeting strategies that combine both Ags and adjuvants are expected to provide the highest specificity of targeting and DC activation and the lowest undesirable side effects.

For translation into humans, Abs against human DEC205 were developed and were shown to be successful in human DEC205 transgenic mice [151]. Human DEC205‑targeting Abs containing NY‑ESO‑1 protein induced human CD8 and CD4 T cell responses in peripheral blood lymphocytes from patients with cancer [152]. In humans, the expression of DEC205 is not restricted to DCs and is present on a wide variety of leukocytes [153]. DEC205 targeting to pDCs and moDCs resulted in uptake, cross‑presentation, and the activation of CD8 and CD4 T cells in vitro [154–156]. In vivo studies using humanized mice indicated that DEC205‑targeting stimulated CD141⁺ DCs and activated CD4 T cells [157, 158]. Using human skin explants, DEC205 Abs were shown to bind to dDCs, and this indicated that skin vaccination could potentially be used in humans [159]. These studies combined have provided a wealth of information on DEC205‑targeted DC vaccination and have paved the way for clinical trials as discussed in the Clinical trials with Abs and ligands section below.

DCIR2

Mouse splenic cDC2s express the CLR DCIR2, which is recognized by the 33D1 Ab. A comparison of DEC205 and DCIR2 targeting revealed that DCIR2 targeting preferentially stimulated CD4 T cells, whereas DEC205‑targeted formulations excelled in CD8 T cell activation [54]. In addition, Ags targeting to DCIR led to T cell–dependent B cell responses [61]. Actually, both DEC205 and DCIR2 targeting stimulated Ab titers against dengue virus, but DEC205 targeting was more protective [160]. These studies suggested that targeting to DCIR2 would not be well suited for the induction of antitumor immunity. However, targeting to DCIR2 did suppress tumor growth in mice challenged with B16–OVA. The vaccination regimen used in this study mainly induced CD4 T cells, only low amounts of CD8 T cells, and also some NK cells [161]. Together, these reports show that targeting to a DC subset less efficient in cross‑presentation can lead to tumor regression and that targeting strategies should not necessarily be limited to one subset. Although mice express multiple DCIR molecules, humans express only one, which is expressed on different DC subsets and B cells. Targeting of human skin and blood cDCs and pDCs in vitro with DCIR Abs resulted in uptake and presentation of Ags and the induction of Ag‑specific CD8 T cells [131, 162]. In humanized mice, vaccination with DCIR‑specific Abs stimulated T cells [158]. However, one important aspect of DCIR is that it contains an ITIM motif and has suppressive effects on Ag targeting [129–131].

CLEC9a/DNGR1

CLEC9a/DNGR1 is predominantly expressed on mouse cDC1 cells and the human equivalent DC subset that expresses CD141/BDCA3 [26–28, 163] (Fig. 1). Mouse studies showed that Ag targeting to CLEC9a induced strong antitumor immunity via the activation of CD8 T cells [27, 28, 164, 165]. Targeting of MUC1 to CLEC9a induced antitumor immunity in mice and specific CD8 T cell activation in human cell culture systems [164]. In addition to CD8 T cell responses, CLEC9a targeting stimulated strong humoral responses, which could be induced, even without adjuvant, in mice and nonhuman primates, although it seems crucial to determine which Ab is used for targeting [166–168].

In vitro studies with human cells demonstrated that CLEC9a is internalized after mAb binding and can lead to Ag presentation to both CD4 and CD8 T cells [169]. In humanized mice, anti‑hCLEC9a Abs targeted specifically to CD141⁺ DCs and resulted in Ag presentation specifically by this subset, whereas anti‑hDEC205 targeted to a wide variety of human leukocytes and was presented by both the CD141⁺ and the CD1c⁺ subsets [170]. These results indicate that CLEC9a may be a suitable candidate for targeting in humans and may elicit both T cell and humoral responses.

Essentially all CLEC9a targeting studies were performed with Abs. CLEC9a binds F‑actin on necrotic cells and that enables DCs to cross‑present dead cells [171–173]. However, until recently, the manufacturing of synthetic ligands remained challenging. Yan et al. [174] performed a detailed analysis of the binding domains of CLEC9a and identified a peptide that interacted with 2 key residues (Asp^{248} and Trp^{250}) of the receptor. Conjugation of that peptide to the immunodominant, OVA epitope significantly enhanced activation of OVA‑specific CD8 T cells by CLEC9a⁺ DCs and decreased metastasis formation in a B16‑OVA melanoma lung‑metastasis model. This was the

CLEC9a DCs and decreased metastasis formation in a B16–OVA melanoma lung metastasis model. This was the first report, to our knowledge, demonstrating a ligand targeting strategy to CLEC9a, and a direct comparative analysis between antiCLEC9a Abs and ligands can clarify which targeting strategy might be more efficient.

CLEC12a

CLEC12a (DCAL - 2, CLL - 1, MCIL, KLRL1) is an inhibitory, ITIM - containing CLR that is predominantly expressed on myeloid cells but can also be found on B cells, CD8 T cells, and NK cells [175–178]. pDCs and mouse cDC1s express CLEC12a to the greatest extent, but it is also expressed on a subset of mouse cDC2s [179, 180]. CLEC12a is less efficient in inducing cytotoxic T cell responses when compared with CLEC9a [167], although a recent study indicated that mature DCs could cross - present CLEC12a - targeted Ags [181]. CLEC12a targeting did also stimulate Ab responses [179]. Because CLEC12a targeting was less efficient than CLEC9a or DEC205 targeting, it was not considered a superior targeting molecule. Nevertheless, CLEC12a targeting on human DCs was shown to lead to efficient cross - presentation and activation of specific CD8 T cells in vitro [182].

Dectin - 1

Dectin - 1 is mainly expressed on mouse cDC2s and not on cross - presenting cDC1s. Consequently, Ab - mediated targeting of OVA to Dectin - 1 in mice induced stronger CD4 T cell and B cell responses and weaker CD8 T cell responses when compared with DEC205 targeting [183]. Targeting of human Dectin - 1 on moDCs with an agonistic anti - hDectin - 1 Ab resulted in the activation of the DCs. Conjugation of Ag to the same Ab resulted in the induction of Ag - specific CD8 T cells [184] and the stimulation of specific Th17 cells [185]. The ligand of Dectin - 1 has been used to target DCs and to induce immunogenicity, which was successful, especially when a hexasaccharide was used. However, in those studies, humoral immune responses and not tumor experiments were reported [186–188]. Dectin - 1 ligand has also been used as an adjuvant or to target adjuvants, and this type of adjuvant delivery might be worthwhile to combine with other (targeted) therapies [189, 190].

Langerin

Langerin is highly expressed on LCs and, to a lesser extent, on dDCs in both mice and man [106, 191–193]. In mice, splenic cDC1s express Langerin at a low level in BALB/c mice but not in Bl6 mice. Ag targeting to Langerin stimulated functional CD4 and CD8 T cell responses, with prolonged presentation to CD8 T cells compared with DEC205 [102]. A study comparing various receptors for targeting on the same DC subset in BALB/c × C57BL/6 F1 mice showed that there was no significant difference among DEC205, Langerin, and CLEC9a targeting, even though CLEC9a had greater expression on cDC1 cells than the other receptors had [194]. Anti–Langerin - OVA–conjugated Abs i.d. injected were taken up by LCs, but, although DEC205 targeting resulted in Ag presentation to T cells in ex vivo skin explants, Langerin targeting did not stimulate T cell responses [195]. Further investigation showed that Langerin⁺ dDCs in mice stimulated CD8 T cell responses, whereas LCs suppressed those CD8 T cell responses [196]. These studies, together with others, suggest that LCs in the mouse have a more tolerogenic role and that dDCs are essential in priming T cell responses, at least during homeostasis.

Although the different types of DC subsets in the skin are very conserved between mice and man, transcriptome analysis showed that human LCs are more related to mouse cDC1 cells, express more cross - presentation machinery - related transcripts, can stimulate naive CD8 T cells, and may have a more - prominent role in CD8 T cell activation than LCs in mice have [97, 197, 198]. Intradermal injection of human Langerin and DEC205 - specific Abs resulted in binding of Langerin Abs to LCs and DEC205 Abs to dDCs, but T cell stimulation vs. tolerance was not investigated [159]. Others showed that Ab - mediated targeting of Ags to Langerin did result in Ag cross - presentation to CD8 T cells, which was enhanced by the TLR - 3 ligand poly - IC [104].

LeY is a ligand for both DC - SIGN and Langerin, and LeY - containing liposomes as well as peptides were targeted to human LCs and dermal DC - SIGN⁺ DCs (Fig. 1). Interestingly, modification of liposomes with LeY enhanced binding to Langerin on LCs but did not result in cross - presentation, whereas it was efficiently cross - presented by DC - SIGN⁺ DCs. In contrast, LeY - modified peptide Ag was efficiently cross - presented by LCs but not by DC - SIGN⁺ DCs. These observations suggest that the combination of the ligand with the Ag formulation determines the uptake, cross - presentation, and subsequent T cell activation efficacy [103]. Altogether, these studies clearly show that Ags directed to Langerin either by Abs or by ligands are cross - presented to CD8 T cells. How effective clinically applicable vaccines are that are targeted to LCs, as compared with dDCs, in terms of priming naive antitumor CD8 T cells, remains to be established.

MR

The MR is not only expressed on macrophage populations and moDCs and CD1c^{+} DCs from the blood but also on endothelial cells and other selected cell subsets [126]. MR appears to direct Ags after uptake to the cross‑presentation pathway [199, 200]. The MR is extensively studied for Ag targeting using ligands, whereas only a few studies have focused on Ab‑mediated Ag targeting (reviewed by [201]). Ab targeting of melanoma‑ and carcinoma‑specific Ags to the MR on human DCs led to Ag presentation and induced T cell activation [131, 152, 202–204]. In comparative studies, both DEC205 and MR targeting could lead to cross‑presentation and CD8 T cell activation against the NY‑ESO‑1 tumor Ags [152].

MR has become the model receptor for ligand targeting and, as such, has been extensively studied for many years. Decades ago, it was shown that s.c. injection of a fusion protein of mucin‑1 (MUC‑1) conjugated to oxidized mannan could prevent outgrowth of MUC‑1–expressing tumors in mice through the activation of tumor‑specific CTL and CD4 T cells [205, 206]. Oxidized mannan matures DCs via TLR4 signaling, and no additional adjuvant is necessary. Since then, clinical phase I–II trials have been performed with oxidized mannan‑MUC1 for adenocarcinoma, which showed that humoral and cellular responses were induced and that protection against recurrence of breast cancer was obtained (see also Clinical trials with Abs and ligands) [207].

Next to protein conjugates, complexes of mannosyl polyethylenimine fused to DNA could transfect human and mouse DCs and were able to activate T cells in an Ag‑specific manner [208]. In addition, direct conjugation of mannose ligands to peptides improved uptake and cross‑presentation in a MR‑dependent manner [209, 210]. Other reports have demonstrated that MR ligand‑coated nanoparticles (liposomes, PLGA particles, dendrimers) containing peptide, tumor lysate DNA, or protein were potent vaccine formulations for the induction of tumor‑specific T cells in vitro and in vivo in mice (Fig. 1) [211–219].

In vitro studies with human cells indicated that immature DCs showed increased binding of liposomes upon addition of mannosylated phosphatidylethanolamine and that changes in ligand valency determined the uptake efficiency by moDCs [220, 221].

Altogether, modification of Ags with MR‑targeting ligands constitutes an effective approach to trigger Ag‑specific antitumor immunity. The biggest challenges now are to discriminate the contribution of the different cell types expressing MR to the induced antitumor responses to optimize current vaccination strategies and the selection of the most‑optimal Ag and formulation combination to test in clinical studies.

DC‑SIGN

DC‑SIGN is highly expressed in moDCs and dermal CD14^{+} DCs and recognizes Le‑type Ags and high mannose carbohydrates [125, 222]. Ab‑Ag conjugates specific for human DC‑SIGN are taken up by moDCs and presented to T cells [223–225] (Fig. 1). Interestingly, different Abs against the carbohydrate recognition domain or neck region of the DC‑SIGN molecule were shown to have different effects on endocytosis, routing, and efficiency in T cell presentation [226], indicating that when evaluating Ab targeting strategies one should realize that is difficult to draw conclusions based on results with a single Ab.

Human DC‑SIGN has multiple homologs in mice, but the mouse distribution patterns differ enormously compared with human DC‑SIGN, as does their glycan specificity and the downstream signaling pathways, activated upon ligand binding [222]. A transgene for DC‑SIGN under the control of the CD11c promoter was generated to enable hDC‑SIGN–targeted mouse vaccination studies [227]. In this hDC‑SIGN transgenic mouse model and in mice with a humanized immune system, Ag targeting via DC‑SIGN Abs induced T cell responses and regression of tumors [228–230]. Injection of nonhuman primates with anti‑DC‑SIGN Abs resulted in labeling of DC‑SIGN–expressing myeloid cells, showing that this model could be used to evaluate the efficacy of DC‑SIGN targeting [231].

In addition to Ab‑mediated Ag targeting, several studies investigated ligand‑mediated tumor Ag targeting to DC‑SIGN. Using the hDC‑SIGN transgenic mice, various Ag formulations bearing ligands for DC‑SIGN have been investigated and have indicated the potency of ligands as targeting moieties. OVA modified with DC‑SIGN‑binding glycans LeX and LeB and OVA‑containing glycoliposomes decorated with LeB were both shown to be effectively taken up by bone marrow–derived DCs from hDC‑SIGN transgenic mice and to elicit activation of murine CD4 and CD8 T cells [232–234]. Similarly, in vivo, a lentiviral vector expressing OVA or tumor Ags, which was modified to express a viral glycoprotein from Sindbis virus specific for DC‑SIGN, stimulated T cell proliferation and induced tumor regression in vivo [235, 236].

and induced tumor regression in vivo [255, 256].

In vitro studies with human cells demonstrated that modification of the recombinant melanoma–associated Ag gp100 with high mannose structures, mainly Man9, enhanced targeting to immature moDCs and increased Ag presentation to gp100 - specific CD4 T cells [237]. A comparable study using MART - 1 coupled to dimannoside (Man α - 6 Man) or a mixture of Le - type blood Ags A and B (LeA/LeB) yielded similar results, confirming the potential of glycotargeting DC - SIGN [238]. Moreover, gp100 and MART - 1 containing liposomes decorated with LeX were shown to be targeted to both moDCs in vitro and CD14+ DC - SIGN+ APCs in situ from human skin explants and could increase Ag presentation to gp100 - and MART - 1–specific CD8 T cell clones [232, 239]. To further strengthen the hypothesis that glycan - engineered liposomes are potent CLR - and DC - targeted vaccines, their ability to prime human primary CD8 T cells was investigated and confirmed by an increase of MART - 1 tetramer - positive CD8 T cells after stimulation of autologous DCs that were loaded with the glycoliposomes [232].

Only a few studies have directly compared Ab and ligand targeting to DC - SIGN. Nanoparticles coated with DC - SIGN ligands and Abs were both efficiently taken up by moDCs and presented to CD8 T cells, but Abs resulted in better CD4 T cell activation [240]. In contrast, in hDC - SIGN transgenic bone marrow–derived DCs, Ab - mediated Ag targeting was more efficient in CD8 T cell activation than in LeB - modified Ag, although both vaccination strategies led to tumor regression [230].

Overall, targeting DC - SIGN with ligands greatly enhances uptake by human and mouse APCs, and if codelivered with tumor Ags, targeted formulations outperform nontargeted formulations in the induction of CD8 and CD4 T cell responses.

MGL

Only 1 MGL gene has been identified in man (CD301, CLEC10a), whereas mice have 2 homologs, MGL1 and MGL2 [241, 242], and this CLR is expressed in immature moDCs, CD1a+ dDCs, and macrophage populations. MGL is an endocytic receptor, and upon MGL binding, Ags are processed and presented to CD4 T cells [243, 244]. Human MGL and mouse homolog MGL2 have high affinities for α - and β - linked *N* - acetylgalactosamine (GalNac, Tn) residues, whereas the mouse homolog MGL1 is specific for LeA and LeX [132, 133]. Most studies have, therefore, used GalNac/Tn residues for the targeting of MGL on DCs and macrophages, and it was shown that GalNac modification of Ags resulted in Ag presentation to CD4 and CD8 T cells using mouse bone marrow–derived DCs [245]. Moreover, targeting MGL - expressing mouse dDCs with MUC1 - Tn proved an efficient strategy for priming of CD4 T cells in vivo and activation of Ab - producing B cells [246]. MUC1 molecules bearing Tn were shown to be delivered to HLA class I and II compartments in DCs [247], and targeting of MGL led to up - regulation of maturation markers on moDCs and stimulation of CD8 T cells [248]. Because targeting human and murine MGL has been shown to induce DC, T cell, and B cell activation, it might encompass a novel approach to design new DC - targeting anti - cancer vaccines. However, more - detailed studies in the context of tumor models are warranted to arrive at a valid evaluation of the efficacy of MGL - targeted vaccines. Moreover, it has to be taken into account that MGL has a very dualistic role since it has also been described to interact with CD45 on T cells and to thereby negatively regulate T cell receptor mediated signaling and cytokine secretion [249]; additionally, MGL binding blocks DC migration to the LNs [250].

Alternative lectins to target

CLRs are not the only lectins that can be targeted for antitumor immune responses. One family of lectins, the Siglecs, has been targeted for antitumor purposes as well [251]. Siglec - 1 (identified as Sialoadhesin and also known as CD169) is expressed on well - defined macrophage populations in lymphoid organs, such as the subcapsular sinus macrophages in the LNs and the metallophilic marginal macrophages in the marginal zone of the spleen, which capture Ags from the lymph fluid and blood, respectively [78, 252, 253]. Targeting Ags to Siglec - 1 in both mice and pigs has been shown to induce strong Ab responses [80, 254, 255], but more important, anti–Siglec - 1 Ab–Ag conjugates can stimulate potent CD4 and CD8 T cell responses and protect against tumors [80, 81, 256]. Siglec - 1+ macrophages may have a direct role in CD8 T cell activation [82, 257], but in addition, transfer of Ags from Siglec - 1+ macrophages to cross - presenting cDC1s in the spleen is involved [81, 82].

Targeting nanoparticles, decorated with an artificial, high - affinity glycan ligands for Siglec - 1 in vitro, led to in vitro T cell proliferation and in vivo to NKT cell activation [258, 259]. These nanoparticles bind very specifically to both human and mouse Siglec - 1, and this may make the translation for human applications easier [259].

CLINICAL TRIALS WITH ABS AND LIGANDS

To date, only a few lectin‑based, DC‑targeted vaccines have been tested clinically in patients with cancer. The first agent to be tested targeted the MR expressed on DCs and macrophages. The agent, CDX‑1307, comprised a human anti‑MR Ab fused to a full‑length human hCG‑β chain protein. hCG‑β is expressed by a variety of common cancer types. Two phase I clinical trials were conducted (CDX‑1307‑01 and CDX‑1307‑02) in patients with locally advanced or metastatic breast, colon, pancreatic, ovarian, or bladder cancer [260]. Patients received CDX‑1307 once every 2 wk for a total of 4 doses either i.d. (CDX‑1307‑01 trial [NCT00709462], n = 57 patients) or i.v. (CDX‑1307‑02 trial [NCT00648102], n = 30). In both phase I trials, a dose escalation of single‑agent CDX‑1307 was performed, and the highest tolerable dose was then coadministered with GM‑CSF (sargramostim, Leukine; sanofi‑aventis, Bridgewater, NJ, USA) or GM‑CSF and poly‑ICLC (Hiltonol; Oncovir, Washington, DC, USA) (both trials). CDX‑1307‑01 additionally included arms in which patients received the fusion protein combined with GM‑CSF and R‑848 (Resiquimod; InvivoGen, San Diego, CA, USA) or all 3 adjuvants. hCG‑β–specific T cells were identified in all cohorts, which included TLR agonist, but no advantage was observed by combining all 3 adjuvants with MR targeting. Humoral anti–hCG‑β responses were greatest in patients receiving all 3 adjuvants; 48 h after i.d. injection, CDX‑1307 could be identified in cells morphologically identified as dDCs or macrophages at the injection site but not in biopsies from a distant site [260]. Clinical responses were observed, with stable disease in 9 patients and mixed responses in 2 patients. Two patients, who had both humoral and cellular responses against the vaccine, had the longest period of stable disease (8.8 and 18.2 mo). Based on these promising phase I results, a phase II trial was initiated in patients newly diagnosed with muscle‑invasive bladder cancer (N‑ABLE study, NCT01094496; Celldex Therapeutics, Hampton, NJ, USA) [261]. Unfortunately, this trial was terminated because of portfolio prioritization by the sponsor after slow accrual.

Another approach to target the MR used oxidized mannan‑MUC1 for patients with carcinoma. In the first clinical trial, 25 patients with advanced metastatic carcinoma were immunized with oxidized mannan‑MUC1, and after 4–8 immunizations, humoral responses were detected in half of the patients, and CD4 and CD8 T cell responses in 20–25% [262]. Next, 3 phase I trials were performed with 41 patients with advanced breast and colon cancer and adenocarcinomas. The mannan‑MUC1 was administered i.m. or i.p., together with cyclophosphamide and was shown not to be toxic. Again, in 60% of vaccinated patients, a strong humoral response was observed with cellular responses in 28% of patients, and there was no added effect observed for the cyclophosphamide [263]. Humoral responses were greater when immunizations were provided i.p. A double‑blind, placebo‑controlled, phase II trial in patients with early stage breast cancer showed long‑term protection against recurrent disease [207]. These studies, although small, suggest that vaccination with oxidized mannan‑MUC1 is harmless and effective in inducing protective immune responses against cancer and should be further investigated in larger trials.

The third reported agent, CDX‑1401, targets the full‑length NY‑ESO‑1 protein to DEC205 expressing APCs [264]. In a phase I study (NCT00948961), CDX‑1401 was administered i.d. to 45 patients with advanced malignancies; of which, 23 patients received the vaccine together with the adjuvants poly‑ICLC and/or Resiquimod (both s.c.). Both humoral and cellular (CD4 and CD8 NY‑ESO‑1–specific) responses were observed, and no dose‑limiting or grade 3 toxicities were reported. Stable disease was observed in 13 patients and tumor shrinkage, based on RECIST criteria, was seen in 2 patients. Maintenance or the induction of an NY‑ESO‑1 T cell response seemed an important factor for reaching stable disease. Interestingly, 6 patients with melanoma received anti‑CTLA4 treatment within 3 mo of the last CDX‑1401 treatment; of which, 4 were reported to reach a partial response or complete response by RECIST 1.1 or irResponse (immune‑related Response) criteria [265], which is greater than the expected 15% response rate for ipilimumab monotherapy. Partial response on immune checkpoint therapy was also reported for 2 patients with non–small cell lung cancer who had received and discontinued CDX‑1401. These very preliminary data are promising and suggest that the combination of a DC‑targeting vaccine with checkpoint inhibitors may have synergistic effects, but the few patients involved obviously call for caution in interpretation of these data.

At the American Society of Clinical Oncology (Alexandria, VA, USA) 2016 annual meeting (Chicago, IL, USA; June 3–7), data were presented on a phase II study (NCT02129075) using CDX‑1401 with poly‑ICLC in patients with advanced melanoma (n = 60) randomized to be pretreated with human Flt3L (CDX‑301) or not [266]. Humoral and cellular responses against NY‑ESO‑1 were observed in both treatment arms but seemed to be stronger or occur at an earlier time point in patients pretreated with CDX‑301. A phase I/II clinical trial (NCT02837991) using CDX‑1401 is currently being conducted in patients with metastatic renal cell cancer.

In addition to the above - described agents, another CLR - targeting agent is currently under clinical investigation in a phase Ib (NCT02387125) and phase II (NCT02609984) study. This agent, CMB305, is a product that combines a DC - SIGN–targeted lentiviral vector encoding NY - ESO - 1 (LV305) and a cancer vaccine, which contains the NY - ESO - 1 protein and a GLA - stable emulsion (GLA - SE), which is a TLR4 - activating adjuvant (G305) (http://www.cancer.gov, ID: 777295). The lentiviral vector alone is also being evaluated clinically in a phase I study (NCT02122861) in NY - ESO - 1–expressing, locally advanced, relapsed, or metastatic tumors, with the option of being combined with anti–PD - 1 (pembrolizumab) treatment for patients with melanoma who had an inadequate response to anti–PD - 1 treatment alone.

These early clinical trials show great promise but also demonstrate the importance of the right selection of Ags and adjuvants in these CLR/cDC - targeted vaccination approaches. Optimal DC activation in skin leading to Th1/CD8 T cell activation requires powerful TLR - L– and/or CD40L - based adjuvants, which should, therefore, be incorporated in CLR - targeted vaccine formulations [267, 268]. The advantage of CD40L - comprising constructs is combined targeting and potent APC activation (adjuvant effect) through CD40/CD40L interaction [269]. In addition to CD40L, other strong DC - activating agents include GM - CSF and TLR - L, of which, the TLR9 agonist CpG seems to perform particularly well. In clinical trials, using only i.d. injection of rhGM - CSF and/or CpG at the site of the primary tumor in patients with early stage melanoma, GM - CSF was shown to activate migratory cDC subsets in the draining sentinel node, whereas CpG injection activated both pDCs and LN - resident cDCs; both adjuvants, alone or combined, induced (re)activation of tumor Ag - specific T cells [113, 115, 116, 270–272]. Other promising adjuvants include TLR2 and TLR3 agonists, which can activate dDCs and the cDC1s, and saponin - based adjuvants, which can improve cross - presentation capacities [267, 273, 274]. Interestingly, topical (but not i.d.) application of the TLR7 agonist imiquimod led to superior CD8 priming by migrated dDCs [275].

The final consideration to be made is whether to selectively target DCs with patients' tumor - specific (and individualized) neoantigens or to use commonly shared tumor Ags, such as cancer testis Ags. The shared Ag approach allows for the generation of "off - the - shelf" products (DNA, mRNA, protein), applicable to multiple patients, to be administered in combination with strong APC - activating adjuvants in vivo for the loading of DCs in situ. The neoantigen approach allows for a "tailor - made" vaccine but does require identification of patient - specific neoepitopes that elicit an Ag - specific T cells response and, thus, requires available tumor tissue to perform genome–wide exome sequencing as well as state - of - the - art, nonsynonymous mutation and neoepitope identification [276–279]. In addition, tumors that have a low mutation burden, might not be suitable for neoantigen - based vaccination strategies and will likely benefit more from the shared Ag approaches [280]. Alternatively, when still present and surgically accessible, the tumor itself could be used as an in vivo source of tumor - specific (shared Ags and neoantigens), by induction of (immunologic) tumor - cell death combined with DC - activating and—possibly targeting—agents.

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

The discovery that various types of DCs exist in mice with different functionalities has led to the search for CLRs that are specifically expressed in cross - presenting DCs that can efficiently stimulate antitumor cytotoxic T cell responses. Tumor Ags linked to Abs and ligands that specifically target those CLRs have shown very strong responses in mouse models. However, because it is doubtful that cross - presentation is a function limited to human cDC1s, other DC subsets, with their specific receptors, could also be efficacious targets for vaccine development. Furthermore, in light of recent observations that multiple T cell interactions with different DC subsets are necessary for providing CD4 T cell help and the generation of optimal CD8 T cell memory [64, 65], selecting a targeting strategy that is restricted to a very specific DC might run the risk of not providing optimal stimulation for long - term immune responses. In this respect, the study by Sehgal et al. [281] is interesting because it compares single and combined DC - SIGN and BDCA - 3 targeting and shows that the combined targeting has synergistic effects on T cell activation. Targeting Ags via carbohydrate ligands that have a broader specificity for several CLRs expressed on several cell types, such as LeY Ags, which can bind to both DC - SIGN and Langerin, or high mannose, which can bind to both DC - SIGN and MR, may have even stronger effects. Promising results have already been observed in clinical studies that target Ags to the MR. Of note, the next step to be tested in clinical studies is the combined application of both in vivo targeting vaccines with checkpoint inhibitors, such as PD - 1 and cytotoxic T lymphocyte–associated protein 4 inhibitors; the first of those studies are underway. The combination of these 2 strategies will be able to activate an antitumor T cell response not yet present in patients with cancer and subsequently to empower those T cells to resist the immunosuppressive effects of the tumor. Because each of these strategies separately already show strong efficacy in preclinical and clinical studies, we

expect the combination will result in synergistic effects and induce potent and long ‐ lasting, antitumor immune responses that will result in regression of tumors and prevent recurrence. Indeed, the possibility that effective vaccination may convert "cold" to "hot" tumors, and so, make them more amenable to immune checkpoint blockade should open up a renaissance and new golden age for tumor vaccines. With our increased knowledge of the various human DC subsets, both in terms of phenotype and functionality, the next generation of DC ‐ targeted vaccines should prove to be more powerful. Specific CLR targeting through high ‐ carbohydrate vaccine formulations may prove instrumental in this regard.

AUTHORSHIP

All authors contributed to the paper, read the draft, and approved the final version. D.v.D. and D.A.S. made the figure.

ACKNOWLEDGMENTS

DISCLOSURES

The authors declare no conflicts of interest.

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