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Review

# Targeted antigen delivery and activation of dendritic cells in vivo: Steps towards cost effective vaccines

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ABSTRACT

During the past decade, the immunotherapeutic potential of ex vivo generated professional antigen presenting dendritic cells (DCs) has been explored in the clinic. Albeit safe, clinical results have thus far been limited. A major disadvantage of current cell-based dendritic cell (DC) therapies, preventing universal implementation of this form of immunotherapy, is the requirement that vaccines need to be tailor made for each individual. Targeted delivery of antigens to DC surface receptors in vivo would circumvent this laborious and expensive ex vivo culturing steps involved with these cell-based therapies. In addition, the opportunity to target natural and often rare DC subsets in vivo might have advantages over loading more artificial ex vivo cultured DCs. Preclinical studies show targeting antigens to DCs effectively induces humoral responses, while cellular responses are induced provided a DC maturation or activation stimulus is co-administered. Here, we discuss strategies to target antigens to distinct DC subsets and to simultaneously employ adjuvants to activate these cells to induce immunity.



## 1. Introduction

Targeted delivery of antigens to DCs in vivo constitutes a promising alternative approach to cell-based therapies. Current cell-based therapies are very costly and only accessible to a limited number of patients. Furthermore, cell-based strategies are limited to DC subsets, or precursors thereof, that can be isolated in sufficient numbers or can be cultured in vitro and distribute poorly following administration to the patient [1]. Cell-based therapies with ex vivo generated DCs have the clear advantages that defined DC subtypes can be directly loaded with antigens, that DCs are stimulated during ex vivo culture without the apparent risk of systemic activation of other immune cells and that the DC activation status can be checked before administration to the patient. Nevertheless, it is questionable whether this will eventually weigh up to the benefit of an "off the shelf" product. In addition, targeted in vivo strategies might benefit from reaching multiple and often rare DC populations in their natural environment. Several DC subsets have now been defined, also in man. They display distinct antigen processing and presenting capabilities and secrete different cytokines, which allow for the design of therapeutic protocols that more precisely control the type of immune response that is initiated upon vaccination. Especially the recognition that specific DC subsets excel in cross-presenting exogenous antigen on MHC class I molecules to activate $CD8^+$ T cells seems critical in DC therapy, which is often harnessed to treat diseases that are difficult to cure without activation of the cellular arm of the immune system.

Over the past decade there have been numerous studies exploring human cells or mouse models showing the efficiency and efficacy of targeted delivery of antigens to DCs. Some of the key studies are shown in the timeline in Fig. 1. The first clinical trials are currently underway to determine safety and proof-of-principle. This review focuses on several important factors that will ultimately determine the outcome of targeted therapies, including the DC subset that is targeted, the expression pattern and biological properties of the target receptors and how or whether adjuvants should be co-administered to boost immune responses.

## 2. DC subsets and their receptors

Various receptors have been identified that mediate binding and uptake of antigen resulting in antigen presentation via MHC. Fc receptors (FcRs) are classical antigen uptake receptors driving antibody-mediated presentation of antigens by antigen presenting cells (APCs). In pioneering studies by Snider et al., bispecific antibodies recognizing both antigen and FcRs are shown to induce

*Abbreviations:* APC, antigen presenting cell; cDC, conventional dendritic cell; CLR, C type lectin receptor; DC, dendritic cell; FcR, Fc receptor; IFN, interferon; LC, Langerhans cell; MPL, monophosphoryl lipid A; MR, mannose receptor; NLR, NOD-like receptor; NLRP3, NLR pryin domain containing 3; pDC, plasmacytoid dendritic cell; PRR, pattern recognition receptor; R848, resiquimod; RLR, retinoic acid inducible gene 1 like receptor; scFc, single chain variable fragment.

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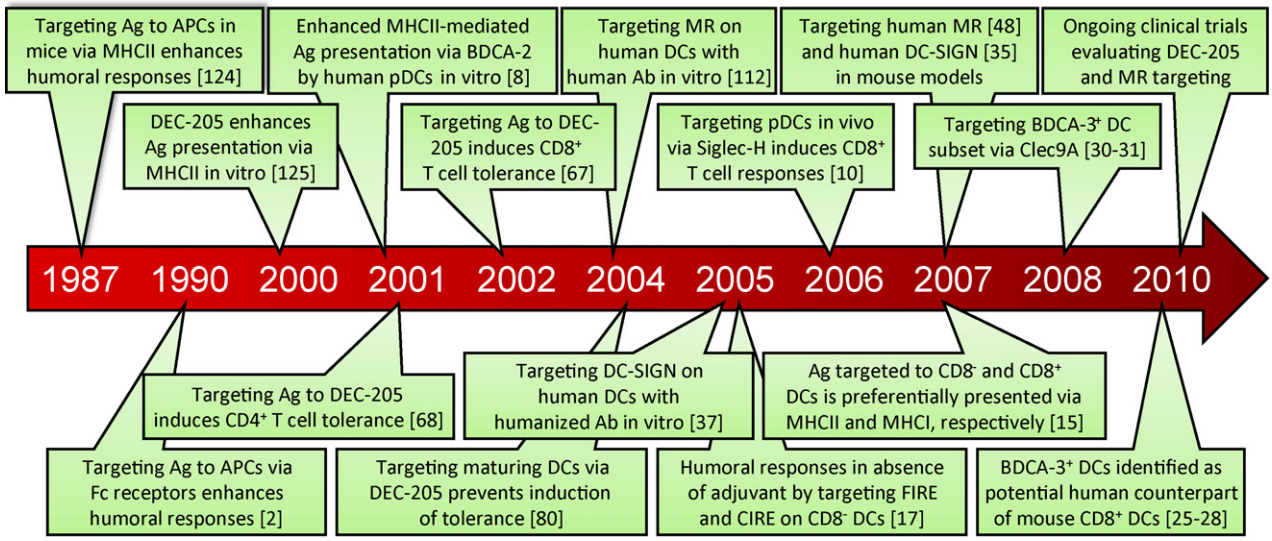


**Fig. 1.** Progress in DC-targeted antigen delivery. The figure depicts a timeline of the past three decades of research on targeted antigen delivery to APCs, showing some of the key studies [124,125].

potent humoral responses [2]. Later, identification of receptors that are more specifically expressed by DCs, or distinct DC subsets, were identified and harnessed for vaccination strategies. The majority of receptors employed for such strategies belong to the family of C-type lectin receptors (CLRs), which bind sugar residues present on pathogens or endogenous proteins [1] (Table 1). Initially, strategies have been developed to produce glycosylated vaccines that are preferentially taken up by professional APCs expressing these CLRs. However, it remains difficult to specifically target a single cell subset with sugars, as they are often recognized by multiple receptors. Lately, there is a shift of interest towards the use of antibodies to increase the specificity of targeting strategies, since it is becoming increasingly evident that reaching specific DC subsets might give control over the type of immune response that is induced. The major DC subsets can be divided into plasmacytoid DCs (pDCs), conventional DCs (cDCs) and Langerhans cells (LCs).

**Table 1**
Expression of various CLRs on human DCs subsets.

| | MR | DEC-205 | DC-SIGN | Langerin | BDCA-2 | Clec9A |
|---|---|---|---|---|---|---|
| moDC[a] | + | + | + | – | – | – |
| Blood CD1c$^+$ | – | + | – | – | – | – |
| Blood CD16$^+$ | – | + | – | – | – | – |
| Blood BDCA-3$^+$ | – | + | – | – | – | + |
| pDC | – | + | – | – | + | – |
| Dermal CD14$^+$ | + | – | + | –[d] | – | ? |
| Dermal CD1a$^+$ | ±[b] | ?[c] | – | –[d] | – | ? |
| LC | – | + | – | + | – | ? |

[a] moDC are monocyte-derived DCs cultured ex vivo in the presence of GM-CSF and IL-4.

[b] There is controversy regarding MR expression in dermal DC subsets. Whereas some studies report MR expression is not expressed on dermal CD1a$^+$ DCs, others report low numbers of MR expressing CD1a$^+$ cells in the upper dermis or high expression levels on many CD1a$^+$ cells [40,118,119].

[c] DEC-205 is expressed in the dermis by a small subset of CD1c$^+$ dermal DCs [120]. Most CD1a$^+$ dermal DCs express CD1c, but is not known whether they co-express DEC-205 [41].

[d] Langerin has long been thought to be exclusively expressed by LCs in skin, and rare Langerin expressing DCs in the dermis were considered to be migrating LC. However, a CD103$^+$/Langerin$^+$ DC subset that is specialized in cross-presentation was recently identified in mouse dermis [22,121–123]. It remains to be established whether a similar Langerin expressing subset is present in human dermis.

### 2.1. pDCs

PDCs are relatively long-lived cells circulating in blood, bone marrow, and peripheral tissues and play an important role in combating viral infections by producing type I interferons (IFNs). Initially, pDCs were considered to be poor antigen presenting cells. It now appears that their antigen presenting abilities are fundamentally different than those of cDCs. In contrast to cDCs, pDCs show continuous production and turnover of MHC-II molecules upon activation, which reduces their ability to present exogenous antigen [3]. Nevertheless, pDCs have been shown to take up and present exogenous antigens derived from apoptotic cells [4], viruses [5], immune complexes [6] and phagocytosed particulates [7]. Initial clinical studies with ex vivo antigen loaded and activated pDCs demonstrate safety and the initiation of antigen specific immune responses (de Vries, personal communication). Targeted delivery of antigens to the CLRs BDCA-2 and DCIR on human pDCs results in antigen presentation in vitro, although both strategies suppress the ability of pDCs to produce type I IFNs [8,9]. Siglec-H represents a pDC-specific endocytic receptor in mice that has been shown to mediate antigen presentation. Targeted delivery of antigen to Siglec-H in vivo results in the activation of CD8$^+$ T cells [10], suggesting pDCs might be explored for targeted vaccination therapies. Taken together, there are a number of surface receptors that may be successfully exploited to target pDCs. Nonetheless, the exact role pDCs play in the initiation of adaptive immune responses will certainly need further clarification before rational approaches to target antigen to this subset can or will be developed.

### 2.2. cDCs

cDCs are specialized antigen processing and presenting cells from myeloid precursors that have a relatively short lifespan. They are found in blood, peripheral tissue and lymphoid tissue and are further subdivided into various subsets. In mice, two major DC subsets are present in lymphoid tissues, the CD8α$^+$ and CD8α$^-$ DCs. CD8α$^+$ DCs express the CLR DEC-205 and are specialized in capturing and cross-presenting antigens derived from apoptotic cells. Moreover, activated CD8α$^+$ DCs produce IL-12p70, which stimulates Th1 responses [11]. CD8α$^-$ DCs express the CLR DCIR2 and are specialized in processing antigens for presentation by MHC-