Designing the Optimal Vaccine: the Importance of Cytokines and Dendritic Cells[#]

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Abstract: Many vaccines existing today provide strong protection against a wide variety of infectious organisms, and these consist of either live attenuated or inactivated microorganisms. Most of these vaccines were developed empirically and there has not been a clear understanding of the immunological principles that contribute to this success. Recent advances in systems biology are being applied to the study of vaccines in order to determine which immunological parameters are the best predictors of success. New approaches to vaccine development include the identification of peptide epitopes and the manipulation of the immune response to generate the most appropriate response. Vaccines are being developed to prevent and/or treat such conditions as cancer and autoimmunity in addition to infectious diseases. Vaccines targeting this diverse group of diseases may need to elicit very different types of immune responses. Recent advances in our understanding of the functions of dendritic cells (DC) and cytokines in orchestrating qualitatively different immune responses has allowed the design of vaccines that can elicit immune responses appropriate for cancer, autoimmunity or infectious organisms. This review will focus on recent advances in the ways DC and cytokines can be used to develop the most appropriate and effective vaccines.

Keywords: Dendritic cells, cytokines, vaccine, immune response, T helper cells.

INTRODUCTION

The development of vaccines for infectious diseases has been one of the success stories in medicine, and devastating diseases such as smallpox have been eradicated thanks to the widespread use of vaccines. The most successful vaccines consist of live-attenuated organisms such as the vaccines against polio, yellow fever and smallpox. These vaccines were designed empirically and we know little of the immunological mechanisms that characterize the most successful of these vaccines. Today vaccines are being developed not only for the prevention of infectious diseases but also for the treatment and prevention of conditions such as autoimmunity and cancer. The goal of vaccination in the cancer or infectious disease setting is the generation of specific immune responses that can induce tumor/pathogen rejection. Conversely, vaccination against autoimmunity should specifically prevent autoimmune tissue destruction. Clearly the nature of the immune response necessary for the rejection of tumor or pathogens is not the same as that needed to prevent autoimmunity or transplant rejection. It is thought that the induction of type 1 immune responses, characterized by interferon (IFN)-γ production and cytotoxic T cells (CTL), is necessary for efficient tumor rejection [1]. In contrast the induction of type 2 immune responses, characterized by interleukin (IL)-4, and the expansion of T regulatory (Treg) cells is beneficial for the prevention and treatment of autoimmunity [2, 3]. In addition, infectious organisms such as HIV, *Plasmodium falciparum* and *Toxoplasma gondii* will not be controlled with the same effector immune responses [4]. HIV vaccines should elicit strong Th1 and CTL responses in order to eliminate infected cells as well as neutralizing antibodies that can prevent infection. Malaria vaccines are complicated by the complex life cycle of the parasite and it may be necessary to induce neutralizing antibodies to the sporozoite stage in order to prevent infection and cell-mediated immunity to target parasites that have invaded the liver [5]. Progress has been made in the identification of epitopes for use in vaccines for many of these conditions [6], but there is now a need to develop new vaccination strategies to ensure that the immune response appropriate for the challenge is induced. It is therefore important to understand in more detail the innate and adaptive responses that are initiated by the most successful of the existing vaccines. A recent study by Querec *et al.* has attempted to do this for the yellow fever vaccine, using the modern day tools of high throughput biological analysis coupled with systems biology and computational modeling [7]. In this study, healthy individuals were vaccinated with the yellow fever vaccine and detailed analysis of the cytokine production, cell surface phenotype and transcriptional activity of peripheral blood mononuclear cells at various time points following vaccination was performed. These studies revealed specific gene expression profiles that were predictive of $CD8^+$ T cell responses and neutralizing antibody responses. Increases in the expression of complement components were found to predict robust $CD8^+$ T cells while the expression of the BLys-BAFF receptor (TNFRSF7) was a key predictor for the B cell re-

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sponses [7]. These studies indicate that key innate immune responses can predict the generation of effective adaptive T and B cell responses following vaccination. Many of these innate responses involve dendritic cells (DC) and the cytokines they produce. DC are the sentinels of the immune system and express a panoply of pattern recognition receptors (PRR) that recognize pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs). These receptors include toll-like receptors (TLR) that can recognize both PAMPs such as LPS [8] and DAMPs such as high mobility group box 1 protein (HMGB1) [9], as well as DAMP-specific receptors such as the receptor for advanced glycation end products (RAGE) [10]. Activation of DC *via* these receptors promotes their migration to draining lymph nodes, increased antigen presentation to T cells and the elaboration of cytokines that drive the differentiation of T cells down specific effector pathways. In this review we will discuss the features of DC and the cytokines they produce that drive specific immune responses and how this knowledge can be harnessed in the design of effective vaccines against infectious, autoimmune and malignant diseases.

DENDRITIC CELLS AND THE CONTROL OF THE IMMUNE RESPONSE

DCs are professional antigen presenting cells (APC) that are uniquely able to activate naïve T cells. In the steady state conventional (c)DC reside in the peripheral tissues where they sample proteins and particulates from the local environment. DCs express receptors such as TLR that recognize molecules expressed by pathogenic organisms as well as endogenous signals of tissue damage [11]. Signaling through these receptors leads to activation and maturation of the DC, resulting in the downregulation of the antigen uptake machinery, upregulation of molecules important for antigen presentation to T cells and migration of the DC from the tissues to the draining lymph nodes where naïve T cells reside. In the lymph node, antigen-specific naïve $CD4^+$ T cells recognizing antigen on DC will be induced to expand and depending on the signals delivered by DC, will differentiate into various effector T cell types. These include T helper (Th)1, Th2, Th17 and Treg subsets, each of which have distinct functions and can be distinguished by the pattern of cytokines they secrete [12, 13]. Activation of naïve $CD8^+$ T cells often requires cross-presentation of antigens, a function that involves the presentation of soluble proteins in the MHC class I pathway for recognition by $CD8^+$ T cells [14], and distinct cytokine profiles also exist for $CD8^+$ T cells. Several DC features determine the nature of specific T cell responses and these include the mechanism by which the antigen was taken up, the cytokines secreted, the level and type of co-stimulatory molecule expression and the dose of antigen presented, in terms of peptide/MHC complexes (Fig. **1**) [15, 16]. Some of these features vary by DC subset and others are influenced by the nature of the invading pathogen or antigens that are taken up [17]. Subsets of DC include both cDC and plasmacytoid (p)DC. pDC circulate in the blood and through lymph nodes and provide early responses to viral infection through the secretion of factors such as type 1 IFN [18-20]. Many of these subsets can be distinguished by the expression of receptors that play important roles in antigen uptake, such as FcR, DC inhibitory receptor (DCIR) and CD205, which, as discussed below, can be targeted in vaccine design. Current vaccine strategies are taking advantage of these features to induce the immune response most likely to be efficacious against the target pathogen.

CYTOKINES AND THE INDUCTION OF EFFECTOR T CELLS

Cytokine production, particularly by DC, plays an important role in defining the type of T cell effector response that is induced [16, 21-23]. IL-12, IFN-α and IFN-γ potently induce type 1 immune responses and IL-4 and thymic stromal lymphopoeitin (TSLP) is important for the induction of type 2 immune responses. The differentiation of T cells into IL-17 producing effector cells requires the presence of IL-6 and TGF-β in mouse and IL-1, IL-6, and TGF-β in human [13, 24, 25], whereas the presence of IL-10 and/or TGF-β results in the differentiation of Treg with suppressor function [26, 27]. It was originally thought that distinct DC subsets produced these differing patterns of cytokines [22, 28]. In particular $CD8^+$ $CD205^+$ DC in mice were identified as the subset that produced high levels of IL-12, whereas the production of high levels IFN-α following viral infection was attributed to pDC [18]. However, more recent data have suggested that DC can be polarized to produce different patterns of cytokine by specific PAMPs binding to their receptors [29]. For example engagement of TLR4 by LPS or TLR3 by double stranded RNA leads to high levels of IL-12 production and Th1 differentiation [29]. In contrast engagement of TLR2-TLR1 heterodimers leads to IL-23 production [30, 31] whereas triggering of TLR2-TLR6 heterodimers with zymosan leads to IL-10 production [32]. An additional layer of complexity is provided by the fact that different sets of PRR are expressed by different DC subsets: for example only pDC in the human express TLR7 and TLR9 [18] thus allowing them to respond to viral infections. This is not the case in mouse where TLR7 and TLR9 are expressed by both cDC and pDC, and this needs to be taken into account when testing vaccine strategies in mouse models. Recent studies have suggested that targeting multiple TLR or PRR simultaneously leads to more robust cytokine production by DC [31, 33]. This makes sense since most pathogens express more than one PAMP and would therefore be expected to engage several different PRRs. In addition to triggering multiple PRR optimal cytokine production can be induced when TLR are engaged in the presence of inflammatory cytokines. In work recently published we and others showed that TLR9 engagement by CpG led to high IL-10 and low IL-12 production by murine DC, but the addition of IFN-γ completely inhibited IL-10 production leading to enhanced IL-12 secretion [34, 35]. The presence of DAMPs, such as HMGB1 and uric acid, as indicators of host cell damage also enhance DC maturation and cytokine production. Thus, DC do not become fully activated unless triggered *via* both a PRR and an inflammatory cytokine or signal of cell damage. The most effective vaccines will likely include TLR and/or PRR ligands that induce DC to adopt the appropriate maturation phenotype and cytokine production profile for the target disease. The choice of these TLR/cytokine combinations will determine the character of the immune response elicited and a few examples are provided in Fig. (**1**).

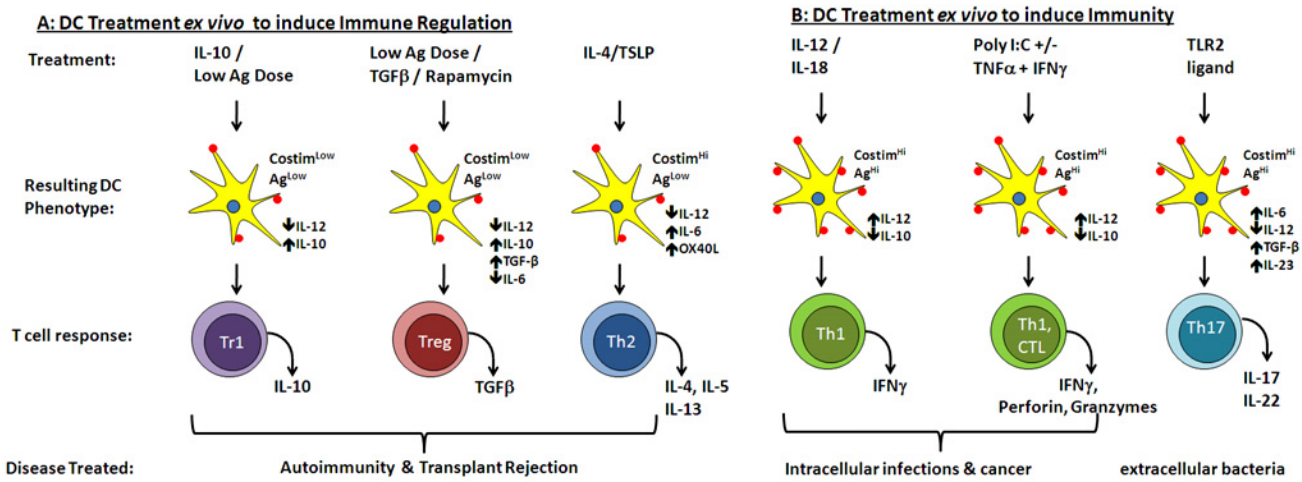

Fig. (1). Examples of *ex vivo* treatment of DC leading to immune regulation (**A**) or immunity (**B**). The treatment can include cytokines such as IL-10, IL-12, IL-4, TLR ligands such as poly I:C, low antigen dose or pharmacological agents such as rapamycin. These treatments change the DC phenotype in terms of co-stimulatory molecules expression and cytokine production as indicated. These result in the differentiation of specific Th subsets such as Tr1, Treg and Th2 (**A**) or Th1, CTL and Th17 (**B**).

Vaccine Adjuvants to Control Cytokine Production

As discussed above, the engagement of PRR on DC or other APC induces the secretion of cytokines that have profound effects on subsequent adaptive T cell responses. This has been exploited by vaccinologists in the design of novel adjuvants to target specific PRR. In animal studies, complete Freund's adjuvant, which contains killed mycobacteria, provides strong stimulation of multiple PRR and is a powerful adjuvant for type 1 immune responses. However, it is too toxic for use in human vaccines and thus more defined adjuvants have been tested for potential use in man. These include TLR4 or TLR9 agonists such as monophosphoryl lipid A (MPL) or CpG respectively [36, 37]. DNA vaccination has the added advantage of combining the adjuvant properties of bacterial CpG in the plasmids encoding the vaccine antigen. Interestingly recent data have suggested that the adjuvant properties of DNA vaccines are TLR9-independent [38] and involve TANK-binding kinase [39]. However, DNA vaccines have not lived up to their early promise and have not stimulated strong immune responses in many cases. This could be due to the route of administration as well as the localization of the expressed protein antigen [40]. We have shown that gene gun immunization with DNA-coated gold particles targets DC in the skin and in order to elicit strong CTL responses the protein should be expressed either in the cytoplasm or in the membrane. Interestingly when the same constructs were given *via* the intramuscular route CTL responses were elicited when the protein was secreted but not if the expression was restricted to the cytoplasm [40]. In addition, different patterns of cytokines were induced depending both on the route of vaccination and cellular localization of the expressed protein. DNA vaccines have also been made more effective by co-administering plasmids that express chemokines known to be important in the recruitment of DC [41]. In these studies plasmids expressing macrophage inflammatory protein (MIP)1-α and Flt-3 ligand were combined with an HIV DNA vaccine. This approach increased the migration of DC to the injection site, which resulted in enhanced protective HIV-specific $CD4^+$ and $CD8^+$ T cell responses [41]. Recent studies have also targeted DNA vaccines to DC to further improve efficacy [42]. Thus DNA vaccines can be made more effective by changing the route of vaccine administration, the cellular localization of the expressed protein and by adding other factors such as chemokines and cytokines.

DC AS VACCINES

Another approach for inducing the appropriate cytokine milieu at the site of vaccination is to generate DC *ex vivo* and expose them *in vitro* to antigens in the presence of TLR ligands or maturation cocktails. This approach has been most extensively used in the case of vaccines with tumor antigens [1, 43] with the aim of inducing longstanding anti-tumor cytotoxic responses (Table **1**). Multiple approaches have been employed, including pulsing DC with peptide, proteins, tumor lysates or live tumor cells in the presence of various cytokine cocktails. DC have also been transduced with plasmids encoding cytokines such as IL-12 and IL- 18 [44] and infected with viral vectors encoding cytokines or tumor antigens [45]. DC vaccines have also been co-administered with tumor cells transduced with cytokines such as GM-CSF [46]. One of the difficulties associated with developing strong immune responses against tumor cells is that the tumor itself secretes immunosuppressive cytokines such as TGF-β [47] and induces the expansion of myeloid-derived suppressor cells [48], which suppress adaptive and innate immune responses. Vaccination strategies have to overcome this immunosuppressive milieu and the fact that individuals with tumors show reduced ability to respond to vaccines. Another concern with this approach is the fact that DC need to traffic to the draining lymph node in order to stimulate T cell responses. Maturing DC *in vitro* with various cytokine cocktails, increases their ability to migrate to lymph nodes [49, 50], but DC are not always able to deliver the required cytokine signals upon arrival at the lymph node due to DC exhaustion [51]. There have been recent improvements in the cocktails used to mature DC for tumor vaccines [52], and these have been shown to generate mature DC capable of secreting large

Table 1. Examples of DC-Based Vaccines used to Induce Immunity or Immune Regulation

DC Vaccines against Cancer				
Vaccine Approach	**Adjuvant or Treatment**	**Target Disease**	**Outcome**	**References**
Ex vivo derived DC given s.c.	Maturation cytokine cocktails, pulsed with tumor antigen	Various cancer types	Improved tumor rejection	[52, 53, 118]
Ex vivo derived DC.	Transduced to express cytokines	Melanoma, glioma	Improved Th1 response	[44, 119-121]
Irradiated tumor cells	Transduced to express cytokines	Various cancers	Increased DC migration and maturation	[46, 122]
Ex vivo derived DC	Transduced to express tumor antigen	Melanoma	Improved Th1 response	[45, 123]
Ex vivo derived DC	Express tumor peptide coupled to MHC class I tracking signals	Lymphoma	Enhanced T cell response and tumor rejection	[86]
In vivo targeting with peptide coupled to anti-CD205 mAb	Poly I:C	Survivin	Strong Th but no CTL response	[96]
In vivo targeting with VLP	Contained tumor antigen HER2Neu	Breast cancer	Prevention of tumor outgrowth	[124]
DC derived exosomes	None	Pancreatic cancer	Activate NK cells	[125]
DC Vaccines against Autoimmunity and Transplant Rejection				
Vaccine Approach	**Adjuvant or Treatment**	**Target Disease**	**Outcome**	**References**
Ex vivo derived DC	$CD86^{Hi}$ DC selected	Type 1 diabetes	Disease prevention; induction of Th2 and Treg	[71, 72, 77]
Ex vivo derived DC	TNF-α (semi-mature)	EAE and EAT	Induction of Treg	[75, 126]
Ex vivo derived DC	TGF-β	EAMG	Disease Prevention. Reduced specific antibody levels.	[61]
Ex vivo derived DC	Pharmacological agents (aspirin, rapamycin, PGE_2, Vit D_3	Organ transplantation, and autoimmunity	Disease prevention; Treg induction	[62-68]
In vivo targeting	Vitamin D_3 and mycophenolic acid	Organ transplantation	Prolonged graft survival; reduced DC maturation	[63, 64]
In vivo targeting with peptide coupled to anti-CD205 mAb	None	Type 1 diabetes	Prevent disease; induce Treg	[102]
In vivo targeting with PLGA beads	Rapamycin	Organ transplantation	reduced DC maturation	[107]
DC-derived exosomes	DC transduced with IDO	Collagen induced arthritis	Suppress DTH and arthritis	[117]
DC Vaccines against Infectious Diseases				
Vaccine Approach	**Adjuvant or Treatment**	**Target Disease**	**Outcome**	**References**
Ex vivo derived DC	Transduced with viral mRNA	HCV, SIV	Virus-specific T cell response	[54, 55]
Ex vivo derived DC	Infected with attenuated virus	Yellow fever	Virus-specific T cell response	[56]

Table 1. contd….

DC Vaccines against Infectious Diseases				
Vaccine Approach	**Adjuvant or Treatment**	**Target Disease**	**Outcome**	**References**
Ex vivo derived DC	Express CMV peptide coupled to MHC class I tracking signals	CMV	Enhanced T cell response	[86]
DNA vaccine – i.m. injection	MIP1-α and Flt3L expressing plasmid	HIV	Increased DC migration, improved T cell response	[41]
In vivo targeting with peptide coupled to anti-CD205 mAb	Anti-CD40 mAb and/ or poly I:C	*Yersinia Pestis,* EBV, *L. Major,* HIV	Strong Th1 and antibody response	[98, 99, 101, 127]
In vivo targeting with peptide coupled to anti-DCIR2 mAb	Poly I:C	*L. Major*	Th2 response	[101]
In vivo targeting with PLGA beads	None	*L. monocytogenes*	Survive lethal infection	[106]
In vivo targeting with VLP	None	HPV, HIV, ebola	DC activation and strong T cell responses	[111-114]
DC derived exosomes	None	*S. pneumoniae*	Survive lethal infection	[116]

amounts of IL-12 and to drive potent Th1 responses [53]. *Ex vivo* generated DC have also been used to vaccinate against infectious diseases such as hepatitis C virus (HCV) [54] and simian immunodeficiency virus (SIV) [55]. In these cases DCs were transduced with mRNA expressing viral proteins, resulting in robust anti-viral immune responses. A recent study examined the ability of DC, infected with the yellow fever vaccine strain, to induce immune responses and found that pretreating the DC with TNF-α protected the DC from the cytopathic effects of the virus. Virus-specific $CD8^+$ T cell responses were induced following co-culture of T cells with the infected DC [56].

DC vaccines have been also been used in the context of autoimmune disease and transplantation [2, 57]. In these cases the tolerogenic properties of DC are exploited by generating DC that either delete autoreactive T cells or induce Treg cells. In the context of transplantation immature DC appear to be the most effective and many approaches have been utilized to induce and maintain this state of immaturity [58]. These include the use of cytokines such as IL-10 and TGF-β [59-61], pharmacological agents such as aspirin [62], vitamin D_3 [63, 64], prostaglandin E_2 [65, 66] and rapamycin [67, 68]. DC cultured in rapamycin are phenotypically immature and are also resistant to further maturation stimuli, which has been attributed to the induction of ST2, a negative regulator of TLR signaling [69]. While there has been some success with immature DC in the context of autoimmunity [70] our own studies have suggested that mature DC are better able to prevent autoimmune disease [71]. In these studies DC were generated from bone marrow cultures with GM-CSF and IL-4 and expressed high levels of MHC and co-stimulatory molecules but secreted low levels of IL-12 when stimulated with TLR ligands or CD40L [72, 73]. This phenotype is similar to semi-mature DC that have been described by several groups [74, 75]. We showed that therapeutic DC induced type 2 cytokine production in treated non-obese diabetic (NOD) mice [71, 76] and more recently we observed that semi-mature DC induce expansion of Treg cells [77]. Furthermore we observed that there was an inverse correlation between the induction of Treg by DC and the strength of the TCR signal as measured by signaling *via* the Akt/mTOR pathway [77]. Interestingly both mature and immature DC could induce Treg expansion provided the appropriate dose of the antigenic peptide was used, and this was observed in three different TCR transgenic systems [77]. The Akt/mTOR signaling pathway has been implicated in the induction of Tregs since it has been shown that inhibition of this pathway results in increased Treg expansion [78, 79]. Indeed low dose antigen has been used in several animal models of autoimmune disease [80, 81] to induce tolerance and this has been attributed to the induction of Treg. Signaling *via* the Akt/mTor pathway represents the culmination of signals received *via* various receptors on the T cell surface including TCR, CD28 and cytokine receptors such as IL-6 and IL-2 [82]. Thus it is possible that immature DC are most effective at inducing Treg in the context of transplantation because they would deliver weak stimulation *via* the TCR to high-affinity alloreactive T cells and induce Treg differentiation, whereas those same T cells would receive strong signals from mature DC which express high levels of MHC and provide additional signaling *via* co-stimulatory molecules and cytokines to result in the expansion of effector cells rather than Treg. In contrast, autoreactive T cells are usually of low affinity and thus mature DC are required in order to trigger the low level of Akt/mTOR signaling that is optimal for Treg induction (Fig. **1**).

TARGETING DC *IN VIVO*

While the generation of DC *ex vivo* provides an attractive way to control the function and phenotype of DC this approach is costly and time-consuming and is not practical for the widespread use of vaccines that are necessary to protect

against infectious diseases. Thus vaccines that target DC *in vivo* are considered to be optimal [83, 84]. As discussed in some detail above it is necessary to ensure that the appropriate DC subset is targeted with the optimal dose of antigen and that the cytokines produced by those DC will drive differentiation of the desired T cell and B cell responses.

DC subsets can be distinguished by the expression of receptors that play important roles in antigen uptake, such as FcR, DCIR and CD205, and these have been used as vaccine targets (Table **1**, Fig. **2**) All of these receptors direct the antigen to specific intracellular compartments that can influence antigen presentation potential, such as cross-presentation [85]. We have shown that the localization of antigenic protein within the cell is very important for the induction of specific immunes responses [40]. In these experiments DNA vaccination with plasmids that targeted protein production to different cellular compartments was performed, strong CTL responses were induced when the protein was cytoplasmic or transmembrane whereas strong antibody responses were induced when the protein was secreted [40]. In another approach, the direct coupling of antigenic peptides to MHC class I trafficking signals was shown to enhance cross-presentation and CTL responses [86]. Antigen uptake receptors have been shown to be important for cross-presentation of antigen to $CD8^+$ T cells [85]. In the mouse, splenic and lymph node DC that express CD205 are specialized in cross-presentation and thus induce strong $CD8^+$ T cell responses [87-89]. In contrast, DCIR-2^+ DC present antigen preferentially to $CD4^+$ T cells and do not effectively cross-present to $CD8^+$ T cells [90]. In human, DCs do not express CD8α and thus the exact human equivalent of the murine $CD8^+$ $CD205^+$ DC has not been clearly defined. A recent study demonstrated that only $CD1c^+$ DC from human peripheral blood or *in vitro*-generated DC were able to cross-present antigen to $CD8^+$ T cells whereas pDC were unable to do so and only presented antigen to $CD4^+$ T cells [91]. It was interesting to note that, in this study, cross-presentation by $CD1c^+$ DC required that the antigen be complexed with specific antibody, thus allowing uptake by FcγR. Other uptake receptors such as DC-SIGN (CD209), Langerin (CD207) and DCIR are type II proteins of the C-type lectin family [43]. A novel C-type lectin (Clec9A) was recently identified on murine $CD8^+$ DC and pDC, and was also found on a subset of human DC [92]. Pathogens binding to these receptors can also influence DC maturation. For example HIV, CMV and several other viruses bind to CD209 and inhibit DC maturation [43, 93], whereas HIV binding to CD207 results in degradation of the virus and reduced transmission to T cells [94].

Several of these receptors have been targeted in vaccine strategies designed to induce specific immune responses [83]. Approaches have included the coupling of antigenic peptides to antibodies specific for certain receptors or to pathogen associated proteins known to bind specifically to uptake receptors. CD205 has been targeted by several groups [87, 95, 96] and in these studies DNA encoding antigenic peptides has been cloned in frame into the anti-CD205 antibody [95, 96] or the peptide itself coupled to the anti-CD205 antibody using biochemical means [87]. This has proved to be a very efficient way of activating specific T cells and extremely small doses of antigen are required. However, in the absence of any stimulus to activate the DC *in situ* targeting antigen to CD205 DC either leads to deletion of antigen-specific T cells [95] or the induction of antigen-specific $Foxp3^+$ Treg [97]. The addition of a TLR ligand such as poly I:C [98] or activating antibody such as anti-CD40 [90, 99] was required to induce strong immune responses. Targeting antigens *via* CD205 in this manner leads to the induction of Th1 responses and effective $CD8^+$ T cells since $CD205^+$ DC are effective at cross-presenting antigen and produce high levels of IL-12 [100]. In contrast targeting antigens, using a similar strategy, to DCIR-2, which is expressed on a different subset of DC leads to the induction of Th2 responses [101]. In both of these cases a DC maturation signal such as poly I:C or anti-CD40 mAb has to be included to induce immunity. This fact has been exploited in the area of autoimmunity and a recent study showed that targeting an islet autoantigen to $CD205^+$ DC led to the deletion of the islet antigen specific T cells [102]. However in the case of the tumor antigen, survivin, even the addition of poly I:C to the survivin-coupled anti-CD205 mAb failed to induce $CD8^+$ T cell responses, although robust $CD4^+$ T cell responses and antibodies were induced [96]. This provides a further example of the challenges facing the development of effective vaccines against tumor antigens.

The recently identified DC marker, Clec9a, can also be used to target DCs *in vivo* [92], and in this case no other DC stimulus is required. Using a model antigen this study showed that coupling the antigen to an anti-Clec9A antibody was sufficient to induce strong Th1, CTL and B cell responses (Fig. **2A**) [92]. Other receptors that been targeted for vaccine purposes include CD11b [103] and mannose receptor [104]. In the case of CD11b, investigators took advantage of a CyA, a adenylate cyclase from *Bordatella pertussis,* that is known to bind to CD11b. Coupling of antigenic peptides to CyA resulted in robust type 1 $CD4^+$ and $CD8^+$ T cell responses in the absence of any additional DC maturation signal [103]. To date most of these approaches have used model antigens, but research is starting in murine models using antigens relevant to infectious disease and autoimmunity (Table **1**). As we learn more about the function and distribution of antigen uptake receptors we will be able to take advantage of their properties to design appropriate new vaccines,

DC are phagocytic cells and the development of nanoparticles, such as liposomes and biodegradable polymers has led to a new approach to vaccine design [105] (Fig. **2B**). Vaccine antigens can be encapsulated within these particles or attached to the surface. A recent study using biodegradable poly(γ-glutamic acid) (PLGA) nanoparticles demonstrated that these particles were taken up by DC *in vivo* and induced potent humoral and cellular immunes responses [106]. The particles were taken up by several DC subsets in the spleen and induced maturation of the DC, cytokine production and type 1 immune responses. Vaccination using these particles coated with a peptide from *Listeria monocytogenes* was able to protect mice from lethal infection [106]. Interestingly if the peptide was encapsulated within the particle the mice were not protected [106], suggesting the rate of degradation and release of antigenic peptide is important [105]. These particles can be coated with various DAMPS or PAMPS in order to provide an added adjuvant effect. A similar approach has been used to develop tolerogenic DC by the delivery of rapamycin, an mTOR inhibitor, encapsulated within PLGA nanoparticles [107]. This approach was found to be more efficient than soluble rapamycin in the modulation of

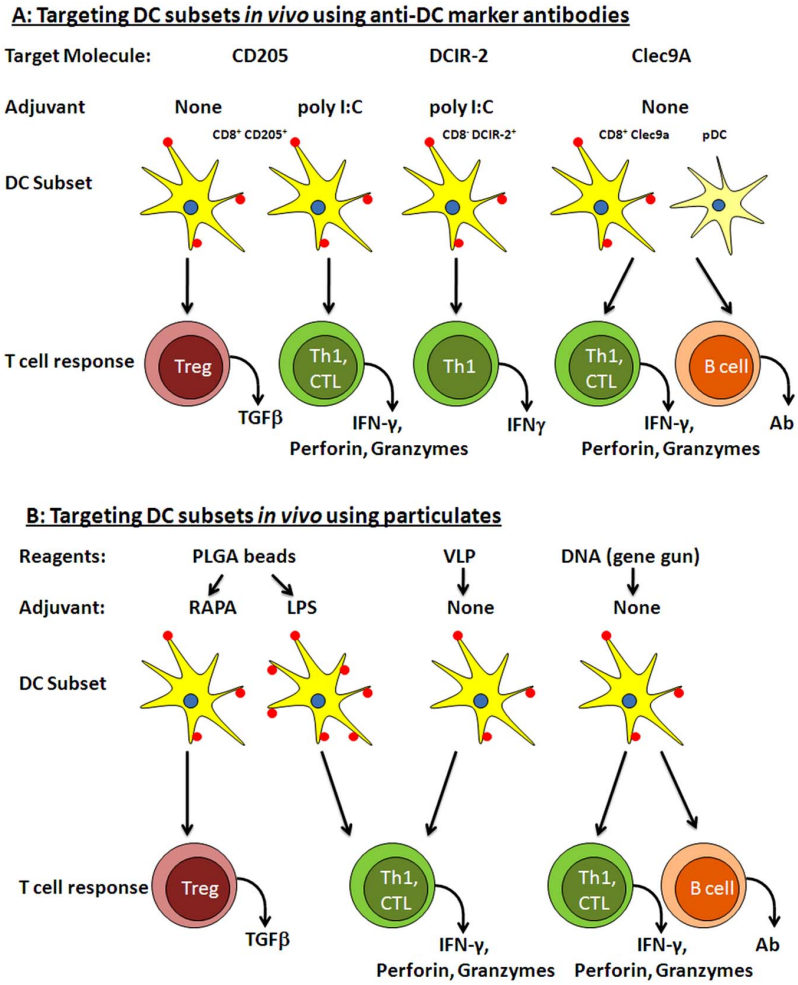

Fig. (2). Examples of *in vivo* targeting of DC using antibodies to specific DC markers (A) or particulates (B). **A**. Three potential target molecules are shown along with the expected immune responses generated. In the case of CD205 and DCIR-2 an additional adjuvant such as poly I:C is required in order to induce immunity, whereas targeting Clec9A alone results in potent immune responses. Tolerance can be induced by targeting antigen to CD205 in the absence of adjuvant. **B**. Three different particulates are shown with the expected immune responses. These responses are influenced by altering the size of the particles and by including adjuvants such as LPS or drugs such as rapamycin. In the case of DNA vaccines immune responses can be influenced by the route of immunization and by the cellular localization of the expressed protein (see text).

DC function [107]. Another approach along these lines has been the use of virus-like particles (VLP) [108], which have been successfully developed for the recently approved human papilloma virus (HPV) vaccine [109]. VLPs consist of the empty capsid of a virus and can be made from the corresponding virus [108], or they can be chimeric and consist of viral or tumor proteins incorporated into a VLP backbone [108]. The size of all of these particles can be varied and it has been shown that distinct DC subsets take up particles based on size [110], such that large particles are taken up by DC at the injection site whereas smaller VLP traffic to the lymph node where they are taken up by resident DC [110]. DC uptake of VLPs results in activation and the stimulation of strong specific T and B cell responses [111-114]. As discussed above this can have an impact on the type of immune response that is elicited. VLP-based vaccines are in development for several infectious diseases and cancer (Table **1**).

DC also release exosomes, which are small nano-sized vesicles that contain immunomodulatry cytokines and mediators. In a recent study DC were treated with diphtheria toxoid (DT) resulting in the release of exosomes than express MHC and co-stimulatory molecules. Administration of these DT-induced exosomes *in vivo* resulted in DT-specific antibody responses [115]. A similar study demonstrated that exosomes from bone-marrow derived DC shared a cross-reactive epitope with a polysaccharide from *Streptococcus pneumoniae.* Mice immunized with these exosomes were protected from lethal pneumococcal infection [116]. DC-derived exosomes can also be used in the context of autoimmune disease especially if the exosomes are derived from DC exposed to cytokines such as IL-10. In a recent study exosomes from DC transduced to express indoleamine-2-3-deoxygenase (IDO) were shown to prevent collagen-induced arthritic in mice [117]. It is not entirely understood how exosomes exert their tolerogenic or immunogenic effects but this is likely to prove a fruitful area of research in the future. DC can thus be targeted using particulate vaccines of various types and the immune response that results will depend on a

variety of factors such as particle size, location of the antigen in or on the particle, the rate of degradation and the addition of adjuvant molecules that induce DC maturation and activation (Fig. **2B**).

CONCLUSIONS

Early success in vaccine development was based on empiricism and we have limited understanding of how to measure and predict the effectiveness of new vaccines that are being developed. The recent study of the yellow fever vaccine [7] provides an approach by which to assess future vaccine efficacy. It is important to understand the type of immune response required to respond to a particular challenge, be it type 1 immune responses for viral infections and cancer, type 2 responses for parasitic infections, type 17 responses for bacterial and fungal infections or regulatory T cell responses to prevent autoimmunity. DC and the cytokines they produce play a key role in driving these immune responses and can be harnessed to induce an effective immune response against the pathogen or disease of choice. A wide array of tools are being developed to target vaccines to specific DC subsets in order to achieve the desired immune response. Major challenges still remain before these approaches can be widely used. These include: 1) defining the appropriate immune response for a particular challenge in terms of which lymphocytes or antibody types will be effective in each context; 2) defining the appropriate antigenic targets and the dose of the vaccinating antigen in order to induce immunity, or tolerance; 3) identifying the appropriate DC subset that should be targeted by the vaccine, either using *ex vivo* or *in vivo* targeting approaches; 4) increasing our understanding of how defined adjuvants work to induce DC maturation and how to combine these for optimal effectiveness; and 5) improving our understanding of the difference between human DC subsets and function and similar populations defined in animal models such as mouse and primate. It is likely that each disease situation and/or pathogen will require a specific approach and the more we can learn about how our most effective vaccines work the better able we will be to design the vaccines of the future.

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