REVIEW

Antibody-targeted vaccines

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The specificity and high affinity binding of antibodies provides these molecules with ideal properties for delivering a payload to target cells. This concept has been commercialized for cancer therapies using toxin- or radionucleotide-conjugated antibodies that are designed to selectively deliver cytotoxic molecules to cancer cells. Exploiting the same effective characteristics of antibodies, antibody-targeted vaccines (ATV) are designed to deliver disease-specific antigens to professional antigen-presenting cells (APCs), thus enabling the host's immune system to recognize and eliminate malignant or infected cells through adaptive immunity. The concept of ATVs has been in development for many years, and recently has entered clinical trials. Early studies with ATVs focused on the ability to induce humoral immunity in the absence of adjuvants. More recently, ATVs targeted to C-type lectin receptors have been exploited for induction of potent helper and cytolytic T-cell responses. To maximize their stimulatory capacity, the ATVs are being evaluated with a variety of adjuvants or other immunostimulatory agents. In the absence of co-administered immunostimulatory signals, APC-targeting can induce antigen-specific tolerance and, thus, may also be exploited in developing specific treatments for autoimmune and allergic diseases, or for preventing transplant rejection. The successful clinical application of this new class of antibody-based products will clearly depend on using appropriate combinations with other strategies that influence the immune system.

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Introduction

After many years of development, monoclonal antibody (mAb)-based therapies have become a critical part of the treatment of patients with autoimmunity, cancer and infectious disease. These therapeutic antibodies all work by exploiting their ability seek out and selectively bind to their target molecules in the patient. Current

approved therapeutic antibody products are targeted directly to pathogens, cancer cells or molecules that contribute to disease. Antibody-targeted vaccines (ATVs), exploit the same targeting properties of antibodies, but are directed to normal cells of the immune system. In the case of ATVs, antibodies are used to deliver a cargo of antigens to professional antigen-presenting cells (APCs) with the goal of inducing or enhancing immunity to the antigen. Although APCs have passive mechanisms for internalizing antigens for processing and subsequent presentation, they are relatively rare cells in the body and this process can be made far more efficient by targeting antigens to cell surface receptors on APCs. Moreover, antibodies specific for endocytic receptors on APCs can lead to antigen accumulation in the intracellular vesicles that are critical for loading appropriate major histocompatibility complex (MHC) molecules required for eliciting effector T-cell responses.

Professional APCs are critical for the induction of potent antigen-specific immune responses. Among APCs, dendritic cells (DCs) are particularly specialized for inducing potent immune responses owing to their high expression of cell surface receptors and a number of complementary molecules that participate in interactions with T lymphocytes (Mellman and Steinman, 2001). DCs also secrete many immune modulators such as cytokines and chemokines to initiate an immune response, which results in the amplification of both cellular and humoral immunity. DCs express on their surface high levels of MHC molecules that bind and display fragments of antigens for recognition by T cells with the appropriate T-cell receptors. Antigens taken up by DCs can be processed and presented by both MHC class I and II molecules to specific CD8⁺ and CD4⁺ T cells, respectively, with the former involving a process called cross-presentation or cross-priming (Bevan, 1976; Rock, 1996; Melief, 2003). This process is critical for induction of cytolytic T-cell (CTL) responses, which are important for effective antiviral and antitumor immunity.

The first studies of highly efficient processing and presentation of antigens targeted to APC-surface molecules were published over 20 years ago. B cells were shown to be very efficient at selectively presenting their specific antigen or antibodies targeted to their cell surface antigen receptors (Chesnut and Grey, 1981; Tony and Parker, 1985). Since that time, a number of other APC cell surface molecules have been used for

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targeted antigen-delivery experiments, and there has also been a tremendous effort in the field of antibody engineering and development, which has led to relatively straightforward approaches to generating a variety of antibody-based vectors. Fusion proteins between antibodies and antigens can now be readily constructed by molecular engineering (Figure 1), and these products can be expressed in well-developed manufacturing processes that can simplify the translation to clinical studies, and provide a viable product development opportunity. The ability to generate targeting antibodies with human sequences, primarily via various applications of genetic engineering technologies, further enhances the applicability of ATVs for human use.

The first efforts at using antibodies to develop vaccination approaches based on the delivery of antigens to APCs, focused on the induction of humoral responses. Using model antigens targeted to MHC molecules and Fc receptors, without adjuvant, led to antigen-specific antibody titers that were comparable to immunizations with antigen emulsified in complete Freund's adjuvant (CFA) (Carayanniotis and Barber, 1987, 1990; Snider and Segal, 1987, 1989; Carayanniotis *et al.*, 1988, 1991; Snider *et al.*, 1990). These seminal studies also demonstrated that robust antibody responses to ATVs could be generated at very low antigen concentrations. In subsequent studies, targeting antigen to CD11c, which is expressed predominantly on DCs in the mouse, resulted in IgG responses that were measurable 5 days after a single immunization without adjuvant, and splenocytes from immunized mice could be successfully fused to create IgG-secreting hybridomas only 10 days after a single immunization (Wang *et al.*, 2000; Berry *et al.*, 2003). ATVs have also been exploited for induction of helper and CTL responses.

A variety of receptors have been exploited for enhancing immune responses using ATVs, with expression profiles that range from relatively APC specific to more widespread patterns (Keler *et al.*, 2005; Tacke *et al.*, 2006). Successfully targeted receptors include family members of the integrins, immunoglobulin superfamily, TNF receptors, complement receptors, and C-type lectin receptors (CLRs). As much of the recent effort has focused on targeting to CLRs, this review will highlight these approaches to vaccine development.

Targeting antigens to CLRs

CLRs are pattern recognition receptors that are abundantly expressed on various APCs populations (Figure 2; Gordon, 2002; Pyz *et al.*, 2006). A characteristic feature shared by many CLRs is their endocytic capacity and the presence of carbohydrate recognition domains (CRDs). The CRDs can selectively bind to glycans associated with microorganisms and lead to rapid internalization (Ezekowitz *et al.*, 1991; Brown *et al.*, 2003; Mansour *et al.*, 2006). This process can initiate effective immune defense, although some pathogens may subvert this pathway and suppress beneficial immune responses (Turville *et al.*, 2003; van Kooyk *et al.*, 2004). Some CLRs that contain a specific triad of acidic amino acids in their carboxyl terminus (e.g. DEC-205) traffic directly to late endosomes that are rich in MHC class II molecules, other CLRs (e.g. mannose receptor (MR)) possess tyrosine-based motifs and rapidly recycle via early endosomes (Mahnke *et al.*, 2000). This diversity in the intracellular routing may have significant impact on the processing and presentation of antigens.

Figure 1 Illustration of a genetically engineered ATV. The illustration shows an example of an expression plasmid for generating an ATV using OVA as a model antigen. The plasmid was transfected into mammalian cells and the ATV purified by affinity chromatography. The ATV and the parental anti-MR mAb were separated by gel electrophoresis and stained with Coomassie Blue. DHFR, dihydrofolate reductase; AMP, ampicillin; NEO, neomycin; OVA, ovalbumin.

Figure 2 CLRs targeted by ATVs.

CLRs may also recognize host-derived cell surface or soluble molecules and in this way play other roles in immune homeostasis and pathology (McKenzie *et al.*, 2002).

Mannose receptor

The MR family of endocytic receptors comprises MR itself, DEC-205, Endo180 and the phospholipase A2 receptor, of which MR and DEC-205 have been best characterized and studied in the context of APC targeting. The extracellular domain of MR comprises

several ligand binding motifs: an N-terminal cysteine-rich (CR) region that recognizes sulfated carbohydrates, a fibronectin-type II (FNII) domain that binds collagens and a tandem array of eight C-type lectin domains (CRDs), only the fourth of which has lectin activity and can thus bind mannose, fucose or N-acetylglucosamine (Stahl, 1992; McGreal *et al.*, 2005). Structural studies have shown that the CR region of both MR and Endo180 bind to the single active CRD (CRD2 for Endo180), which suggests a role in regulating ligand binding activities of these molecules (Boskovic *et al.*,

2006). These ligands can be found on both pathogens and host-derived molecules (Allavena *et al.*, 2004).

In addition to the expression of MR on macrophages where it was initially identified, MR expression has been demonstrated on immature DCs, subsets of endothelial cells and some other cell types (Stahl, 1992; Uccini *et al.*, 1997). Among DC populations, MR expression is abundant on mouse bone marrow-derived DC, human monocyte-derived DCs and interstitial DCs, such as dermal DCs (Sallusto *et al.*, 1995; Uccini *et al.*, 1997). MR is not expressed by Langerhans cells in the epidermis or by plasmacytoid DCs (Mombaas *et al.*, 1999; Guo *et al.*, 2000). Interestingly, MR-expressing DCs are found in the epidermis of patients with active dermatitis, suggesting that these MR-expressing APCs may be mobilized to inflammatory sites (Wollenberg *et al.*, 2002), or MR expression may be induced locally by inflammatory mediators. Either way they may be involved in the endocytosis and clearance of micro-organisms and serum glycoproteins, as well as locally presenting antigens derived from these entities. Although an endocytic receptor, MR alone is not able to initiate phagocytosis (Le Cabec *et al.*, 2005) and consequently plays an important role in uptake and presentation of soluble, but not cell-associated antigens (Burgdorf *et al.*, 2006).

Ligands containing mannan or mannose have been successfully used in various forms to direct antigens to APCs, thus generating more efficient immune responses (Tan *et al.*, 1997; Agnes *et al.*, 1998; Apostolopoulos *et al.*, 2000; Keler *et al.*, 2004). The use of MR ligands for targeting APCs has been widely applied for enhancing antigen-specific immunity, but these ligands are not specific for MR, thus the contribution of MR relative to other mannan-binding receptors, such as DC-specific intracellular adhesion molecule (ICAM)-grabbing non-integrin (DC-SIGN), to the enhanced immune responses remains to be established. The use of CLR-specific antibodies is now permitting the investigation of immune responses mediated by the individual receptors.

Studies by our laboratory have demonstrated that targeting of tumor antigens to the MR on monocyte-derived DCs using a human MR-specific mAb can efficiently sensitize antigen-specific T cells which display MHC class I- and class II-restricted activity (He *et al.*, 2004; Ramakrishna *et al.*, 2004). Importantly, the MR-targeted vaccines elicited CTL responses to multiple epitopes, which resulted in specific lysis of histocompatibility leukocyte antigen-matched tumor cells naturally expressing the antigen. Results obtained using confocal microscopy demonstrated that the internalized anti-MR mAb localized to MHC class I-containing vesicles, but not to MHC class II-containing vesicles, both in peripheral and in internal vesicular compartments.

Using a transgenic mouse model in which the human MR is expressed under the control of its own promoter, MR-targeted ATVs have demonstrated efficient targeting to MR-expressing cells *in vivo*. Moreover, these ATVs were only effective in potent immune responses and antitumor activity in the MR-tg mice. Strong

cellular immunity was dependent on concomitant administration of adjuvants (He *et al.*, 2006).

Clinical development of ATVs has in fact been initiated with a fusion protein (CDX-1307) composed of an MR-specific IgG1 κ human mAb (clone B11) and the beta (β) chain of human chorionic gonadotropin (hCG). hCG- β is overexpressed in a variety of common cancers including those of the colon, lung, pancreas, esophagus, breast, bladder, cervix, stomach and prostate (Triozi and Stevens, 1999; Butler and Iles, 2003). Elevated hCG- β serum levels and/or tissue expression have been shown to be independent predictors of disease outcome and are associated with a more aggressive disease course in renal, colorectal, bladder and pancreatic cancers. To our knowledge, this is the first investigation of ATVs in human clinical trials. CDX-1307 is currently in two dose escalation phase I clinical trials for patients with incurable cancers. The primary distinction between the studies is the route of administration, which includes intradermal and intravenous to compare the local versus systemic vaccine effects.

DEC-205

DEC-205 is a CLR belonging to the MR family (Kato *et al.*, 2006). DEC-205 contains 10 CRDs for which the specific ligands remain to be identified. The human and murine DEC-205 share 77% identity, and their expression is primarily restricted to DCs and thymic cortical epithelium, although human DEC-205 can also be detected on peripheral T and B cells (Kato *et al.*, 2006). A distinguishing feature of DEC-205 is its high-level expression on DCs in the T-cell areas of lymphoid organs, suggesting a role in regulating T-cell responses. DEC-205 is internalized by means of coated pits and vesicles, and traffics to endosomal compartments that are rich in MHC class II molecules (Jiang *et al.*, 1995). In contrast, MR expression on DCs is primarily restricted to interstitial DCs such as dermal DCs, and traffics to early endosomes that do not contain MHC class II (Mahnke *et al.*, 2000).

The potential of antigen targeting to DEC-205 has been pioneered by Drs Steinman and Nussenzweig at Rockefeller University. Initial studies demonstrated the ability of rabbit antibodies specific for mouse DEC-205 to be presented to reactive T-cell hybridomas 100-fold more efficiently than irrelevant rabbit IgG (Jiang *et al.*, 1995). Further studies showed that the DEC-205 cytosolic domain drives a receptor-mediated endocytosis that involves efficient recycling through late endosomes (Mahnke *et al.*, 2000). In addition, compared to the MR cytosolic domain, the intracellular routing by DEC-205 was more efficient in presenting antigen to CD4⁺ T cells. Priming antigen-specific CD4 T cells with ATVs specific for the DEC-205 receptor on DCs *in vivo* resulted in remarkable expansion of antigen specific, MHC class II restricted T cells (Hawiger *et al.*, 2001). These studies demonstrated that targeting to DEC-205 was far more efficient for induction of T-cell responses than immunogenic peptide mixed with CFA. However, to maintain the T-cell response and prevent antigen-specific anergy,

activation signals provided by an agonistic anti-CD40 mAb were required.

Recently, the use of ATVs to elicit protective and therapeutic immunity has been addressed using tumor cells or pathogens that were modified to express ovalbumin (OVA) as a model antigen (Bonifaz *et al.*, 2004b). Targeting OVA to DEC-205 in mice resulted in exceptional cross-presentation of antigen to CTL (Bonifaz *et al.*, 2002). Similar to the effect observed on CD4 T cells, induction of sustained immunity required co-administration of activating signals in the form of anti-CD40 mAbs or CFA (Bonifaz *et al.*, 2002, 2004b). Similar data has also been obtained with DEC-205 targeted survivin (Charalambous *et al.*, 2006) and human immunodeficiency virus antigens (Trumpfheller *et al.*, 2006). Bonifaz *et al.* (2004b) also demonstrated the induction of substantial antitumor effects when mice were challenged with OVA expressing syngeneic tumor cells. The protection in this model required CD8 T cells and demonstrated that cross-presentation *in vivo* was essential for the protective responses. Despite the use of local immunization, the targeted vaccine resulted in systemic antigen loading of DCs and MHC class I presentation that persisted for at least 2 weeks. Furthermore, administration of this ATV 1 week post-tumor challenge resulted in a more significant delay of tumor progression compared to other popular methods of vaccinating.

Mahnke *et al.* (2005) developed DEC-205 targeted ATVs with the melanoma antigen tyrosinase-related protein-2 and used a vaccination strategy combined with cytosine-guanosine oligonucleotide (CpG) oligodeoxynucleotides to investigate the effect on tumor challenge with the melanoma cell line B16. The DEC-205-targeted vaccination protected mice against tumor growth and substantially slowed the growth of previously implanted B16 cells. Protection was mediated by melanoma-specific CD4 and CD8 T-cell responses.

A DEC-205-targeted ATV was also investigated in a model of viral infection (Bonifaz *et al.*, 2004a). Subcutaneously delivered DEC-205-targeted OVA in combination with the agonist anti-CD40 mAb induced immunity against infection with OVA modified vaccinia virus administered intranasally. Mice vaccinated with the ATV had significantly reduced virus titers and symptoms compared to controls.

In most of the systems studied thus far, induction of antigen-specific immunity with DEC-205-targeted ATVs was dependent on concomitant delivery of a DC activation/maturation signal. In fact, antigens targeted to DEC-205, in the absence of activating stimuli, can induce profound peripheral T-cell tolerance (Hawiger *et al.*, 2001; Bonifaz *et al.*, 2002). These findings are consistent with the concept that DCs play a critical role in maintaining tolerance to self-antigens in the absence of DC activation signals (Heath and Carbone, 2001). Thus, in the absence of DC activation, APC targeting may also be exploited therapeutically to develop specific immunosuppressive ATVs that have useful application for treatment of autoimmune diseases, transplantation or allergy. This is discussed briefly at the end of this review.

DC-SIGN

DC-specific ICAM-grabbing non-integrin (DC-SIGN) is the founding member of a family of type II membrane-spanning CLRs that is expressed by DCs and macrophages (Geijtenbeek *et al.*, 2000). It has been most actively investigated for its role in binding and promoting human immunodeficiency virus infection of T cells (Geijtenbeek *et al.*, 2000; Bashirova *et al.*, 2001; Pohlmann *et al.*, 2001), but also has a number of other ligands, including interactions with ebola, measles and herpes viruses and *Neisseria gonorrhoeae* and *meningitides*. In addition, DC-SIGN is important in the interaction between DCs and T cells through its ICAM-3 binding function. The expression of DC-SIGN is particularly well suited to ATV approaches as the receptor is expressed at high levels by multiple DC populations that can contribute to T-cell priming and activation. Similar to MR, DC-SIGN is an endocytic receptor that can mediate efficient antigen presentation through different ligands, and more recently using a novel humanized antibody, hD1 (Schjetne *et al.*, 2002; Tacke *et al.*, 2005; Dakappagari *et al.*, 2006).

Studies by Tacke *et al.* (2005) used keyhole limpet hemocyanin (KLH)-conjugated to hD1 to investigate internalization, trafficking and presentation of KLH. The DC-SIGN targeted ATV was rapidly internalized and subsequently routed to the lysosomal compartments. DCs pulsed with the ATV enhanced the proliferation of autologous peripheral blood lymphocytes (PBLs) from KLH vaccinated patients at a 100-fold lower concentration than KLH-pulsed DCs. The hD1-KLH ATV also elicited KLH-specific T cells from unvaccinated donors, and blocking antibodies to MHC molecules suggested that the ATV induced proliferation of T cells recognizing KLH epitopes in the context both MHC class I and MHC class II. Consistent with the previous studies using MR and DEC-205, these studies were also performed in the context of the strong DC activation and maturation stimuli, lipopolysaccharide. Whether or not targeting DC-SIGN will trigger tolerance in the absence of DC-activating agents is currently not known.

Other CLRs

Different APCs express a number of other CLRs that are also beginning to be explored for their utility in APC-targeted antigen delivery (e.g. Dectin-1, Dectin-2, BDCA-2, DCIR, DCAR, Langerin and MGL). With emerging data on signaling activities of these different molecules, either direct or via their interactions with other activating receptors (e.g. Toll-like receptors (TLR) or Fc receptors), it is likely that some could offer different opportunities for therapeutic applications. Two examples will be discussed briefly in this section.

Dectin-1

DC-associated C-type lectin-1 (Dectin-1) is an NK cell receptor-like molecule expressed by APCs and some

other myeloid cells that lacks CRDs but acts as a major receptor for β -glucans, and thus recognizes and can endocytose a number of fungal species, including yeast and *Aspergillus* (Ariizumi *et al.*, 2000; Brown, 2006). Unlike other CLRs, Dectin-1 has an activating ITAM motif in its cytoplasmic tail, and cross-linking this receptor leads to APC activation and pro inflammatory mediator production (Gantner *et al.*, 2003; Brown, 2006), as well as synergistic interaction with other innate signaling molecules such as TLR2 (Gantner *et al.*, 2003). Dectin-1 can also signal to regulate innate immune responses through activation of the Syk tyrosine kinase and subsequent production of interleukin (IL)-2 and IL-10 (Rogers *et al.*, 2005).

In the mouse, Dectin-1 is expressed by CD8 α -CD11b⁺ DCs in spleen, lymph nodes and in skin (dermal DCs) and a recent study by Carter *et al.* (2006b) has shown efficient induction of both T cell (CD4 and CD8) and antibody responses by antigen delivered with a Dectin-1-targeting antibody. They also compared responses to Dectin-targeted antigen with those triggered with a DEC-205-targeting antibody, that targets CD8 α ⁺CD11b⁻ DCs and found qualitatively different outcomes. Dectin-1 produced stronger antibody and CD4 T cell responses, whereas DEC-205 was much better at stimulating CD8 T-cells. This data supports the concept of different applications for different targeting molecules, although it is also possible that the differences are due to inherent differences between the two antibody-antigen conjugates used (e.g. relative affinity). As evidenced by studies of different antibodies to MR, different receptor epitopes can also trigger quite different responses (Chieppa *et al.*, 2003).

Dectin-2

Dectin-2 is a myeloid-lineage restricted cation-dependent carbohydrate-binding receptor that can recognize fungal hyphae and has a particular selectivity for high mannose structures (McGreal *et al.*, 2006). Its expression is upregulated in macrophage lineage cells under activating inflammatory conditions (Sato *et al.*, 2006), and although it appears to lack any cytoplasmic signaling domains it can couple with FcR γ to trigger innate immune responses. Unlike Dectin-1-targeting data discussed above, anti-Dectin-2-coupled antigen has been shown to efficiently induce CD8 T-cell responses (Carter *et al.*, 2006a).

Immunosuppressive ATVs for autoimmune and allergic disorders

The immune response to antigen is a tightly regulated process that, under normal physiological conditions, serves to protect against pathogens while minimizing effects on self tissues. However, autoimmune diseases and allergies can develop when failure of control mechanisms leads to chronic and excessive immune activation. Autoimmune diseases such as multiple sclerosis and type I diabetes, result from inappropriate

(often Th1-biased) immune responses to self-tissues, whereas allergic disorders are due to uncontrolled Th2-biased responses to exogenous antigens.

Most current treatments for autoimmune and allergic disorders are largely directed at providing symptomatic relief and do not tackle the underlying mechanisms driving these chronic pathologies. Ideally, treatments should be targeted selectively to modulate the antigen responses specifically driving disease pathology, as these should be most effective and could be able to re-establish appropriate control mechanisms (e.g. regulatory T cells) to provide long-term disease modification (Larche and Wraith, 2005). The successes achieved over the past few years in the treatment of certain allergies using different allergen-based immunotherapeutics has provided proof of concept that long-term antigen-specific disease modification is achievable, and mechanistic studies have indicated that successful therapy does indeed involve amplification of regulatory mechanisms (Larche *et al.*, 2006). However, these treatments are still mainly individually tailored and involve many visits to specialist clinics over a number of years – so there is clearly a desire for improved products in this arena as well as for products that take a similar approach for autoimmune disease treatments, albeit targeting different immune mechanisms.

Finkelman *et al.* (1996) were the first to demonstrate immune tolerance induction via antibodies targeting DCs. They showed that mice treated with the rat IgG2b anti-DC antibody 33D1 developed both T- and B-cell hyporesponsiveness to subsequent challenge with rat IgG2b antigen. In these same studies, a different DC-specific antibody (the hamster anti-CD11c antibody N418) stimulated antibody responses rather than inducing tolerance, suggesting that DC surface molecules may differ in their tolerogenic potential. A number of different APC-targeting approaches have since been explored for their potential at triggering or reinforcing immune tolerance mechanisms as a basis for immunotherapeutics. This is particularly well exemplified by the studies of Steinman and co-workers referred to earlier, where antigen targeting to DC surface DEC-205 *in vivo* in the absence of DC maturation signals induced active and specific immune tolerance to the antigen (Steinman *et al.*, 2003). These studies with model antigens have since been followed up in models of autoimmune diseases. Hawiger *et al.* (2001) used a spontaneous onset transgenic model of type I diabetes, whereas Bruder *et al.* (2005) used an experimentally induced acute encephalitis system (EAE), a model of some features of multiple sclerosis. In both settings, treating with a DEC-205-targeted version of the relevant antigen (in the absence of adjuvant) before disease onset or induction induced a marked suppression of disease symptoms and pathology. Mechanistically, active immune suppression involving the amplification of regulatory T-cell function is thought to be involved in controlling pathologic effector responses (Steinman *et al.*, 2003; Maksimow *et al.*, 2006). Whether or not such strategies will be capable of re-inducing tolerance of pre-primed immunity or of suppressing ongoing

pathogenic effector responses, which will be required for the development of successful therapeutic approaches, remains to be determined. Clearly, with respect to the use of ATVs in therapy of cancer or infectious diseases inadvertent induction of immune suppression needs to be avoided through the use of suitable DC-activating agents (Steinman *et al.*, 2003; Maksimow *et al.*, 2006).

Whether or not other APC surface molecules can also be targeted for the induction of immune tolerance is currently unclear. Alveolar macrophages express MR which has been shown to be involved in suppressing local inflammatory responses (Zhang *et al.*, 2005) and cross linking of MR on DCs by certain antibodies has been shown to elicit an anti-inflammatory, immunosuppressive program of cytokine secretion, including high IL-10 and suppressed IL-12 (Chieppa *et al.*, 2003). Such immunosuppressive responses may have direct relevance to allergic responses in the airways, since the DC binding and uptake of the major dust-mite allergen Der p1 has been shown to involve MR (Deslee *et al.*, 2002). Recent studies with the macrophage galactose-type C-type lectin (MGL), which is another endocytic receptor also expressed by DCs, have shown that this molecule has suppressive interactions with T cells through its binding to CD45 (van Vliet *et al.*, 2006), and another CLR, DCAL-2, has also been shown to have immunoregulatory properties (Chen *et al.*, 2006). In addition to peripheral immune suppression, APC-targeting may also be able to induce central (thymus) T-cell tolerance to peptide antigens (Schjetne *et al.*, 2005). Finally, recent disease model studies have explored the potential of using Fc receptor targeting to deliver immunomodulatory approaches for the treatment of rheumatoid arthritis (van Vuuren *et al.*, 2006; Wenink *et al.*, 2006).

Further studies of immunosuppressive APC-targeting approaches will no doubt advance our overall understanding of the mechanisms controlling the balance between immune effector and regulatory responses. Such knowledge will not only help advance potential specific treatments for autoimmune and allergic disorders, it will also have an impact on the development of ATVs for cancer and infectious disease.

Conclusions

APCs have a variety of receptors that mediate the sampling of antigens (both self and non-self) to integrate the innate with adaptive immune responses. In particular, CLRs are abundantly expressed on various APC populations and via the signal motifs in their cytoplasmic domains are able to route antigens to endosomal compartments that are important for antigen processing and presentation. However, most vaccination strategies still rely on the local uptake of antigen. The use of CLR-specific antibodies provides a novel way to exploit these pathways for induction of antigen-specific immunity using the beneficial properties of antibodies that can provide systemic delivery. In fact, preclinical models have demonstrated the efficacy of this strategy using a variety of experimental systems. From a development perspective,

Figure 3 Illustration of ATV-mediated immune responses. ATV can elicit potent immunity when combined with appropriate APC activation, but in the absence of concomitant activation of the APC, targeted delivery of antigen may lead to tolerance. APC, antigen-presenting cell; TLR, toll-like receptor.

ATVs are a practical approach relative to some of the more personalized vaccine technologies. These products can be generated as fusion proteins containing an antigen-genetically linked to the APC-binding human mAb. Therefore, the manufacturing, purification and characterization of these products are based on standardized procedures developed for mAbs, and consequently the regulatory requirements for such well-characterized products are understood. In contrast to traditional antibody approaches, antigens used in ATVs can be intracellular targets. These antigens may consist of either small disease-specific peptides or entire proteins. Moreover, multiple antigens or epitopes defined from different antigens for inducing broader immune responses can be readily assembled into one construct.

Preclinical studies with ATVs have made it apparent that additional signals such as those afforded by adjuvants are likely to be important to elicit sustained immunity in humans (Figure 3). Therefore, at least in this context, ATVs will need to be combined with agents that elicit complementary activation signals. Such signals may be provided by a number of immunostimulatory agents that are actively being investigated in the clinic, such as TLR agonists and cytokines. Although there has been less progress to date, the use of ATVs in the absence of activation signals, and perhaps with additional immune suppression, is an attractive opportunity for developing antigen-specific immune suppression.

Although the ATV technology has been in preclinical development for two decades, only recently has the first candidate molecule entered human clinical trials. Based on strong preclinical data with multiple approaches, additional ATV programs for oncology, infectious disease, and pathogens relevant to biodefense programs are likely to enter clinical development in the near future. In summary, antigen targeting to APCs using antibodies provides a specific and efficient antigen delivery platform for direct *in vivo* application. The addition of immune modulators to APC targeting is a critical feature of ATVs which may be required to tip the balance towards tolerance or immunity.

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References

- Agnes MC, Tan A, Jordens R, Geluk A, Roep BO, Ottenhoff T *et al.* (1998). Strongly increased efficiency of altered peptide ligands by mannosylation. *Int Immunol* **10**: 1299–1304.
- Allavena P, Chieppa M, Monti P, Piemonti L. (2004). From pattern recognition receptor to regulator of homeostasis: the double-faced macrophage mannose receptor. *Crit Rev Immunol* **24**: 179–192.
- Apostolopoulos V, Pietersz GA, Gordon S, Martinez-Pomares L, McKenzie IF. (2000). Aldehyde-mannan antigen complexes target the MHC class I antigen-presentation pathway. *Eur J Immunol* **30**: 1714–1723.
- Ariizumi K, Shen GL, Shikano S, Xu S, Ritter III R, Kumamoto T *et al.* (2000). Identification of a novel, dendritic cell-associated molecule, dectin-1, by subtractive cDNA cloning. *J Biol Chem* **275**: 20157–20167.
- Bashirova AA, Geijtenbeek TB, van Duijnhoven GC, van Vliet SJ, Eilering JB, Martin MP *et al.* (2001). A dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin (DC-SIGN)-related protein is highly expressed on human liver sinusoidal endothelial cells and promotes HIV-1 infection. *J Exp Med* **193**: 671–678.
- Berry JD, Licea A, Popkov M, Cortez X, Fuller R, Elia M *et al.* (2003). Rapid monoclonal antibody generation via dendritic cell targeting *in vivo*. *Hybrid Hybridomics* **22**: 23–31.
- Bevan MJ. (1976). Cross-priming for a secondary cytotoxic response to minor H antigens with H-2 congenic cells which do not cross-react in the cytotoxic assay. *J Exp Med* **143**: 1283–1288.
- Bonifaz L, Bonnyay D, Mahnke K, Rivera M, Nussenzweig MC, Steinman RM. (2002). Efficient targeting of protein antigen to the dendritic cell receptor DEC-205 in the steady state leads to antigen presentation on major histocompatibility complex class I products and peripheral CD8+ T cell tolerance. *J Exp Med* **196**: 1627–1638.
- Bonifaz LC, Bonnyay DP, Charalambous A, Darguste DI, Fujii S, Soares H *et al.* (2004a). *In vivo* targeting of antigens to maturing dendritic cells via the DEC-205 receptor improves T cell vaccination. *J Exp Med* **199**: 815–824.
- Bonifaz LC, Bonnyay DP, Charalambous A, Darguste DI, Fujii S, Soares H *et al.* (2004b). *In vivo* targeting of antigens to maturing dendritic cells via the DEC-205 receptor improves T cell vaccination. *J Exp Med* **199**: 815–824.
- Boskovic J, Arnold JN, Stilson R, Gordon S, Sim RB, Rivera-Calzada A *et al.* (2006). Structural model for the mannose receptor family uncovered by electron microscopy of Endo180 and the mannose receptor. *J Biol Chem* **281**: 8780–8787.
- Brown GD. (2006). Dectin-1: a signalling non-TLR pattern-recognition receptor. *Nat Rev Immunol* **6**: 33–43.
- Brown GD, Herre J, Williams DL, Willment JA, Marshall AS, Gordon S. (2003). Dectin-1 mediates the biological effects of beta-glucans. *J Exp Med* **197**: 1119–1124.
- Bruder D, Westendorf AM, Hansen W, Prettin S, Gruber AD, Qian Y *et al.* (2005). On the edge of autoimmunity: T-cell stimulation by steady-state dendritic cells prevents autoimmune diabetes. *Diabetes* **54**: 3395–3401.
- Burgdorf S, Lukacs-Kornek V, Kurts C. (2006). The mannose receptor mediates uptake of soluble but not of cell-associated antigen for cross-presentation. *J Immunol* **176**: 6770–6776.
- Butler SA, Iles RK. (2003). Ectopic human chorionic gonadotropin beta secretion by epithelial tumors and human chorionic gonadotropin beta-induced apoptosis in Kaposi's sarcoma: is there a connection? *Clin Cancer Res* **9**: 4666–4673.
- Carayanniotis G, Barber BH. (1987). Adjuvant-free IgG responses induced with antigen coupled to antibodies against class II MHC. *Nature* **327**: 59–61.
- Carayanniotis G, Barber BH. (1990). Characterization of the adjuvant-free serological response to protein antigens coupled to antibodies specific for class II MHC determinants. *Vaccine* **8**: 137–144.
- Carayanniotis G, Skea DL, Luscher MA, Barber BH. (1991). Adjuvant-independent immunization by immunotargeting antigens to MHC and non-MHC determinants *in vivo*. *Mol Immunol* **28**: 261–267.
- Carayanniotis G, Vizi E, Parker JM, Hodges RS, Barber BH. (1988). Delivery of synthetic peptides by anti-class II MHC monoclonal antibodies induces specific adjuvant-free IgG responses *in vivo*. *Mol Immunol* **25**: 907–911.
- Carter RW, Thompson C, Reid DM, Wong SY, Tough DF. (2006a). Induction of CD8+ T cell responses through targeting of antigen to Dectin-2. *Cell Immunol* **239**: 87–91.
- Carter RW, Thompson C, Reid DM, Wong SY, Tough DF. (2006b). Preferential induction of CD4+ T cell responses through *in vivo* targeting of antigen to dendritic cell-associated C-type lectin-1. *J Immunol* **177**: 2276–2284.
- Charalambous A, Oks M, Nchinda G, Yamazaki S, Steinman RM. (2006). Dendritic cell targeting of survivin protein in a xenogeneic form elicits strong CD4+ T cell immunity to mouse survivin. *J Immunol* **177**: 8410–8421.
- Chen CH, Floyd H, Olson NE, Magaletti D, Li C, Draves K *et al.* (2006). Dendritic-cell-associated C-type lectin 2 (DCAL-2) alters dendritic-cell maturation and cytokine production. *Blood* **107**: 1459–1467.
- Chesnut RW, Grey HM. (1981). Studies on the capacity of B cells to serve as antigen-presenting cells. *J Immunol* **126**: 1075–1079.
- Chieppa M, Bianchi G, Doni A, Del Prete A, Sironi M, Laskarin G *et al.* (2003). Cross-linking of the mannose receptor on monocyte-derived dendritic cells activates an anti-inflammatory immunosuppressive program. *J Immunol* **171**: 4552–4560.
- Dakappagari N, Maruyama T, Renshaw M, Tacken P, Figdor C, Torensma R *et al.* (2006). Internalizing antibodies to the C-type lectins, L-SIGN and DC-SIGN, inhibit viral glycoprotein binding and deliver antigen to human dendritic cells for the induction of T cell responses. *J Immunol* **176**: 426–440.
- Deslee G, Charbonnier AS, Hammad H, Angyalosi G, Tillie-Leblond I, Mantovani A *et al.* (2002). Involvement of the mannose receptor in the uptake of Der p 1, a major mite allergen, by human dendritic cells. *J Allergy Clin Immunol* **110**: 763–770.
- Ezekowitz RA, Williams DJ, Koziel H, Armstrong MY, Warner A, Richards FF *et al.* (1991). Uptake of *Pneumocystis carinii* mediated by the macrophage mannose receptor. *Nature* **351**: 155–158.
- Finkelman FD, Lees A, Birnbaum R, Gause WC, Morris SC. (1996). Dendritic cells can present antigen *in vivo* in a tolerogenic or immunogenic fashion. *J Immunol* **157**: 1406–1414.
- Gantner BN, Simmons RM, Canavera SJ, Akira S, Underhill DM. (2003). Collaborative induction of inflammatory responses by dectin-1 and Toll-like receptor 2. *J Exp Med* **197**: 1107–1117.
- Geijtenbeek TB, Torensma R, van Vliet SJ, van Duijnhoven GC, Adema GJ, van Kooyk Y *et al.* (2000). Identification of

- DC-SIGN, a novel dendritic cell-specific ICAM-3 receptor that supports primary immune responses. *Cell* **100**: 575–585.
- Gordon S. (2002). Pattern recognition receptors: doubling up for the innate immune response. *Cell* **111**: 927–930.
- Guo M, Gong S, Maric S, Misulovin Z, Pack M, Mahnke K *et al.* (2000). A monoclonal antibody to the DEC-205 endocytosis receptor on human dendritic cells. *Hum Immunol* **61**: 729–738.
- Hawiger D, Inaba K, Dorsett Y, Guo M, Mahnke K, Rivera M *et al.* (2001). Dendritic cells induce peripheral T cell unresponsiveness under steady state conditions *in vivo*. *J Exp Med* **194**: 769–779.
- He LZ, Crocker A, Vitlae L, O'Neill T, Clynes R, Keler T. (2006). Targeting antigens to the human mannose receptor expressed in transgenic mice elicits potent CD4 and CD8 responses *in vivo*. *Proc Amer Assoc Cancer Res* **47**: 334c–335c.
- He LZ, Ramakrishna V, Connolly JE, Wang XT, Smith PA, Jones CL *et al.* (2004). A novel human cancer vaccine elicits cellular responses to the tumor-associated antigen, human chorionic gonadotropin beta. *Clin Cancer Res* **10**: 1920–1927.
- Heath WR, Carbone FR. (2001). Cross-presentation, dendritic cells, tolerance and immunity. *Annu Rev Immunol* **19**: 47–64.
- Jiang W, Swiggard WJ, Heufler C, Peng M, Mirza A, Steinman RM *et al.* (1995). The receptor DEC-205 expressed by dendritic cells and thymic epithelial cells is involved in antigen processing. *Nature* **375**: 151–155.
- Kato M, McDonald KJ, Khan S, Ross IL, Vuckovic S, Chen K *et al.* (2006). Expression of human DEC-205 (CD205) multilectin receptor on leukocytes. *Int Immunol* **18**: 857–869.
- Keler T, He L, Graziano RF. (2005). Development of antibody-targeted vaccines. *Curr Opin Mol Ther* **7**: 157–163.
- Keler T, Ramakrishna V, Fanger MW. (2004). Mannose receptor-targeted vaccines. *Expert Opin Biol Ther* **4**: 1953–1962.
- Larche M, Akdis CA, Valenta R. (2006). Immunological mechanisms of allergen-specific immunotherapy. *Nat Rev Immunol* **6**: 761–771.
- Larch M, Wraith DC. (2005). Peptide-based therapeutic vaccines for allergic and autoimmune diseases. *Nature Medicine* **11**: S69–S76.
- Le Cabec V, Emorine LJ, Toesca I, Cougoule C, Maridonneau-Parini I. (2005). The human macrophage mannose receptor is not a professional phagocytic receptor. *J Leukoc Biol* **77**: 934–943.
- Mahnke K, Guo M, Lee S, Sepulveda H, Swain SL, Nussenzweig M *et al.* (2000). The dendritic cell receptor for endocytosis, DEC-205, can recycle and enhance antigen presentation via major histocompatibility complex class II-positive lysosomal compartments. *J Cell Biol* **151**: 673–684.
- Mahnke K, Qian Y, Fondel S, Brueck J, Becker C, Enk AH. (2005). Targeting of antigens to activated dendritic cells *in vivo* cures metastatic melanoma in mice. *Cancer Res* **65**: 7007–7012.
- Maksimow M, Milunien M, Marttila-Ichihara F, Jalkanen S, Hanninen A. (2006). Antigen targeting to endosomal pathway in dendritic cell vaccination activates regulatory T cells and attenuates tumor immunity. *Blood* **108**: 1298–1305.
- Mansour MK, Latz E, Levitz SM. (2006). Cryptococcus neoformans glycoantigens are captured by multiple lectin receptors and presented by dendritic cells. *J Immunol* **176**: 3053–3061.
- McGreal EP, Miller JL, Gordon S. (2005). Ligand recognition by antigen-presenting cell C-type lectin receptors. *Curr Opin Immunol* **17**: 18–24.
- McGreal EP, Rosas M, Brown GD, Zamze S, Wong SY, Gordon S *et al.* (2006). The carbohydrate-recognition domain of Dectin-2 is a C-type lectin with specificity for high mannose. *Glycobiology* **16**: 422–430.
- McKenzie EJ, Su YP, Martinez-Pomares L. (2002). The mannose receptor, a Bi-functional lectin with roles in homeostasis and immunity. *Trends Glycosci Glycotechnol* **14**: 273–283.
- Melief CJ. (2003). Mini-review: regulation of cytotoxic T lymphocyte responses by dendritic cells: peaceful coexistence of cross-priming and direct priming? *Eur J Immunol* **33**: 2645–2654.
- Mellman I, Steinman RM. (2001). Dendritic cells: specialized and regulated antigen processing machines. *Cell* **106**: 255–258.
- Mommaas AM, Mulder AA, Jordens R, Out C, Tan MC, Cresswell P *et al.* (1999). Human epidermal Langerhans cells lack functional mannose receptors and a fully developed endosomal/lysosomal compartment for loading of HLA class II molecules. *Eur J Immunol* **29**: 571–580.
- Pohlmann S, Baribaud F, Doms RW. (2001). DC-SIGN and DC-SIGNR: helping hands for HIV. *Trends Immunol* **22**: 643–646.
- Pyz E, Marshall AS, Gordon S, Brown GD. (2006). C-type lectin-like receptors on myeloid cells. *Ann Med* **38**: 242–251.
- Ramakrishna V, Trembl JF, Vitale L, Connolly JE, O'Neill T, Smith PA *et al.* (2004). Mannose receptor targeting of tumor antigen pmel17 to human dendritic cells directs anti-melanoma T cell responses via multiple HLA molecules. *J Immunol* **172**: 2845–2852.
- Rock KL. (1996). A new foreign policy: MHC class I molecules monitor the outside world. *Immunol Today* **17**: 131–137.
- Rogers NC, Slack EC, Edwards AD, Nolte MA, Schulz O, Schweighoffer E *et al.* (2005). Syk-dependent cytokine induction by Dectin-1 reveals a novel pattern recognition pathway for C type lectins. *Immunity* **22**: 507–517.
- Sallusto F, Cella M, Danieli C, Lanzavecchia A. (1995). Dendritic cells use macropinocytosis and the mannose receptor to concentrate macromolecules in the major histocompatibility complex class II compartment: down-regulation by cytokines and bacterial products. *J Exp Med* **182**: 389–400.
- Sato K, Yang XL, Yudate T, Chung JS, Wu J, Luby-Phelps K *et al.* (2006). Dectin-2 is a pattern recognition receptor for fungi that couples with the Fc receptor gamma chain to induce innate immune responses. *J Biol Chem* **281**: 38854–38866.
- Schjetne KW, Thommesen JE, Fredriksen AB, Lunde E, Sandlie I, Bogen B. (2005). Induction of central T cell tolerance: recombinant antibodies deliver peptides for deletion of antigen-specific (CD4⁺)8⁺ thymocytes. *Eur J Immunol* **35**: 3142–3152.
- Schjetne KW, Thompson KM, Aarvak T, Fleckenstein B, Sollid LM, Bogen B. (2002). A mouse C kappa-specific T cell clone indicates that DC-SIGN is an efficient target for antibody-mediated delivery of T cell epitopes for MHC class II presentation. *Int Immunol* **14**: 1423–1430.
- Snider D, Segal D. (1989). Efficiency of antigen presentation after antigen targeting to surface IgD, IgM, MHC, Fc gamma RII, and B220 molecules on murine splenic B cells. *J Immunol* **143**: 59–65.

- Snider DP, Kaubisch A, Segal DM. (1990). Enhanced antigen immunogenicity induced by bispecific antibodies. *J Exp Med* **171**: 1957–1963.
- Snider DP, Segal DM. (1987). Targeted antigen presentation using crosslinked antibody heteroaggregates. *J Immunol* **139**: 1609–1616.
- Stahl PD. (1992). The mannose receptor and other macrophage lectins. *Curr Opin Immunol* **4**: 49–52.
- Steinman RM, Hawiger D, Liu K, Bonifaz L, Bonnyay D, Mahnke K *et al*. (2003). Dendritic cell function *in vivo* during the steady state: a role in peripheral tolerance. *Ann NY Acad Sci* **987**: 15–25.
- Tacken PJ, de Vries IJ, Gijzen K, Joosten B, Wu D, Rother RP *et al*. (2005). Effective induction of naive and recall T-cell responses by targeting antigen to human dendritic cells via a humanized anti-DC-SIGN antibody. *Blood* **106**: 1278–1285.
- Tacken PJ, Torensma R, Figdor CG. (2006). Targeting antigens to dendritic cells *in vivo*. *Immunobiology* **211**: 599–608.
- Tan MC, Mommaas AM, Drijfhout JW, Jordens R, Onderwater JJ, Verwoerd D *et al*. (1997). Mannose receptor-mediated uptake of antigens strongly enhances HLA class II-restricted antigen presentation by cultured dendritic cells. *Eur J Immunol* **27**: 2426–2435.
- Tony HP, Parker DC. (1985). Major histocompatibility complex-restricted, polyclonal B cell responses resulting from helper T cell recognition of antiimmunoglobulin presented by small B lymphocytes. *J Exp Med* **161**: 223–241.
- Triozi PL, Stevens VC. (1999). Human chorionic gonadotropin as a target for cancer vaccines. *Oncol Rep* **6**: 7–17.
- Trumpfheller C, Finke JS, Lopez CB, Moran TM, Moltedo B, Soares H *et al*. (2006). Intensified and protective CD4+ T cell immunity in mice with anti-dendritic cell HIV gag fusion antibody vaccine. *J Exp Med* **203**: 607–617.
- Turville S, Wilkinson J, Cameron P, Dable J, Cunningham AL. (2003). The role of dendritic cell C-type lectin receptors in HIV pathogenesis. *J Leukoc Biol* **74**: 710–718.
- Uccini S, Sirianni MC, Vincenzi L, Topino S, Stoppacciaro A, Lesnoni La Parola I *et al*. (1997). Kaposi's sarcoma cells express the macrophage-associated antigen mannose receptor and develop in peripheral blood cultures of Kaposi's sarcoma patients. *Am J Pathol* **150**: 929–938.
- van Kooyk Y, Engering A, Lekkerkerker AN, Ludwig IS, Geijtenbeek TB. (2004). Pathogens use carbohydrates to escape immunity induced by dendritic cells. *Curr Opin Immunol* **16**: 488–493.
- van Vliet SJ, Gringhuis SI, Geijtenbeek TB, van Kooyk Y. (2006). Regulation of effector T cells by antigen-presenting cells via interaction of the C-type lectin MGL with CD45. *Nat Immunol* **7**: 1200–1208.
- van Vuuren AJ, van Roon JA, Walraven V, Stuij I, Harmsen MC, McLaughlin PM *et al*. (2006). CD64-directed immunotoxin inhibits arthritis in a novel CD64 transgenic rat model. *J Immunol* **176**: 5833–5838.
- Wang H, Griffiths MN, Burton DR, Ghazal P. (2000). Rapid antibody responses by low-dose, single-step, dendritic cell-targeted immunization. *Proc Natl Acad Sci USA* **97**: 847–852.
- Wenink MH, van den Berg WB, van Riel PL, Radstake TR. (2006). Fc gamma receptor mediated modulation of dendritic cells as a potential strategy in the battle against rheumatoid arthritis. *Neth J Med* **64**: 103–108.
- Wollenberg A, Mommaas M, Oppel T, Schottdorf EM, Gunther S, Moderer M. (2002). Expression and function of the mannose receptor CD206 on epidermal dendritic cells in inflammatory skin diseases. *J Invest Dermatol* **118**: 327–334.
- Zhang J, Tachado SD, Patel N, Zhu J, Imrich A, Manfrulli P *et al*. (2005). Negative regulatory role of mannose receptors on human alveolar macrophage proinflammatory cytokine release *in vitro*. *J Leukoc Biol* **78**: 665–674.