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Review Article

Targeting Vaccines to Dendritic Cells

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Dendritic cells (DC) are specialized antigen presenting cells (APC) with a remarkable ability to take up antigens and stimulate major histocompatibility complex (MHC)-restricted specific immune responses. Recent discoveries have shown that their role in initiating primary immune responses seems to be far superior to that of B-cells and macrophages. DC are localized at strategic places in the body at sites used by pathogens to enter the organism, and are thereby in an optimal position to capture antigens. In general, vaccination strategies try to mimic the invasiveness of the pathogens. DC are considered to play a central role for the provocation of primary immune responses by vaccination. A rational way of improving the potency and safety of new and already existing vaccines could therefore be to direct vaccines specifically to DC. There is a need for developing multifunctional vaccine drug delivery systems (DDS) with adjuvant effect that target DC directly and induce optimal immune responses. This paper will review the current knowledge of DC physiology as well as the progress in the field of novel vaccination strategies that directly or indirectly aim at targeting DC.

KEY WORDS: Dendritic cells; vaccine; formulation; targeting; review; drug delivery.

INTRODUCTION

The ultimate goal of vaccination is the stimulation of a specific immune response and the induction of a long lasting immunologic memory to protect against subsequent disease. For a successful vaccination the vaccine antigens have to be presented by APC that activate the effector cells of the immune defense, the naïve T-cells and the B-cells.

At least two signals are required for efficient T-cell stimulation by the APCs. The first signal is the presentation of antigenic peptide fragments on the surface of APCs in the context of MHC class I and class II for the recognition by the T-cell receptors (TCR) on T-cells. Co-stimulatory molecules on the APC surface that are recognized by receptors on the T-cell surface provide the second signal. Examples of important co-stimulatory molecules on the APC surface are the B7 family (CD80, CD86) and CD40 that are recognized by CD28 and CD40 ligand on T-cells. Non-professional APCs lack co-stimulatory signaling and can therefore not stimulate effector T-cells sufficiently. It is believed that in the absence of appropriate co-stimulatory signals, TCR recognition of peptides presented on MHC leads to anergy, which would constitute a mechanism of tolerization to self-antigens.

The traditional view has for long been that endogenous antigen peptide fragments generated in the cytoplasm of cells (e.g., by virus infection) by the proteasome are presented by MHC Class I molecules and stimulate cytotoxic T lymphocytes (CTL). Specific Class I restricted $CD8^+$ T-cells can recognize cells presenting the specific antigen on MHC class I on the surface and be induced to exert cytotoxic effector functions leading to lysis of the target cell. Peptide fragments of exogenous protein acquired from outside the cell are presented by MHC Class II molecules and stimulate helper T-cell responses. T-helper cells activate B-cells to become antibody-secreting plasma cells. However, recently evidence for cross talk between these two pathways was suggested since certain types of exogenous antigens also can be presented by MHC class I molecules (1).

Three types of APCs exist: DC, macrophages and B-cells. Of these three cell types, only DC are efficient stimulators of primary immune responses and a subsequent establishment of immunologic memory. This review will therefore focus solely on DC and their importance for successful vaccination.

Many new subunit vaccines based on peptides, proteins and DNA are poorly immunogenic and need to be administered together with an adjuvant to stimulate a sufficient immune response to eliminate pathogens (2). Development of efficient vaccines has for many years been hampered by the lack of adjuvants. Alum has until recently been the only adjuvant approved for use in humans. Progress in the develop-

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ABBREVIATIONS: APC, Antigen Presenting Cells; CTL, Cytotoxic T Lymphocyte; CTLA-4, Cytotoxic T-Lymphocyte Antigen 4; DC, Dendritic Cells; DDS, Drug Delivery System; ER, Endoplasmatic Reticulum; FcγR, Fcγ Receptor; Gb3, Globotriacylceramid; GM-CSF, Granulocyte Macrophage Colony Stimulating Factor; IC, Immuno Complexes; IL, Interleukin; ISCOM, Immune Stimulating Complexes; LC, Langerhans Cells; MIIC, MHC class II Compartments; MHC, Major Histocompatibility Complex; MMR, Macrophage Mannose Receptor; TAP, Transporter associated with Antigen Processing; TCR, T-cell receptor; TGF, Transforming Growth Factor; TNF, Tumor Necrosis Factor.

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ment of vaccines is thus dependent on the discovery and development of new adjuvants and delivery methods that can improve the potency and the safety of existing and new vaccines. Several new approaches are on their way to fulfill this aim.

DC have been characterized as nature's adjuvant due to their ability to initiate primary immune responses. It therefore seems rational to focus vaccine development more on exploiting the unique capacities of DC. Effective future vaccines might well be those that directly target DC. DC can present extracellular antigens by the MHC class I pathway resulting in the killing of target cells and subsequent clearance of disease. This makes them putative vaccination vehicles for so diverse diseases as cancer, autoimmune diseases and infectious diseases.

DENDRITIC CELL PHYSIOLOGY

DC are generated from hematopoietic progenitors in the bone marrow that differentiate into precursors circulating in the blood and the lymph. These precursors migrate to the peripheral tissues where they will reside ready for the encounter with antigens. "Danger signals" (see later section) trigger migration to secondary lymphoid organs, where antigen specific T-cells are activated to initiate an immune response.

Immature Dendritic Cells

Circulating DC precursors can be recruited to sites of antigen deposits by chemokines, released by tissue damage. Expression of several chemokine receptors (CCR1, 2, 4, 5, and 6, and CXCR1 and 4, reviewed in (3)) makes DC able to migrate toward gradients of chemokines (e.g., MIP1α, RANTES, MCD3, MIP5, MCP, reviewed in (3)). DC also express adhesion molecules for entrance to peripheral tissues (4). In the tissues, the DC exist in a stage called immature DC. The efficient, continuous sampling of antigens in the peripheral tissues by the immature DC is one of the reasons why they become potent APCs on maturation.

High sampling of the antigen content of the tissue environment is achieved in two ways: The localization of the DC and a high phagocytic activity. Immature DC are mainly found in areas with potential antigen entry; the skin epidermis (Langerhans cells (LC)), interstitial DC in non-lymphoid tissues (heart, liver, kidney, connective tissue), the spleen, the thymus, the blood, the germinal centers and the T-cell areas of the lymph nodes and mucosal surface associated DC (review by Hart (5)). This localization maximizes the uptake of infectious material.

Immature DC capture antigens through several uptake pathways. Extracellular fluid and solutes are taken up by macropinocytosis (6–8). Large pinocytotic vesicles are formed, and large volumes of fluid can be taken up. Sallusto *et al.* (6) estimated the uptake rate of one DC to be approximately 1000–1500 μm^3 per h corresponding to one cell volume per hour. The huge uptake serves to concentrate antigens in DC and makes the cells efficient in presenting soluble antigens present in nanomolar to picomolar concentrations. Aquaporins in DC are believed to mediate transport of water out of DC to maintain intracellular water pressure homeostasis (9).

DC can also take up antigens by receptor mediated endocytosis. They express two types of the C-type lectin receptors, the macrophage mannose receptor (MMR) (6) and DEC-205 (10). Ligands for MMR are mannose-coated particles such as yeast and bacteria, but the receptor also has specificity for other types of sugar residues. Conjugation of mannose residues to antigens like proteins and peptides has been shown to increase their uptake by DC (11). DEC-205 appears to be specifically expressed on DC and is therefore interesting in the context of vaccine targeting. However, ligands for DEC-205 have not been identified yet. Furthermore, two types of Fcγ receptors (FcγR) mediate endocytosis (7). The Type I receptor (CD64) and Type II (CD32) receptor mediate the uptake of immune complexes (IC) and opsonized particles, respectively.

The third uptake mechanism used by DC is phagocytosis. Particulates are taken up by this mechanism, e.g., latex particles (12–13), microbes (12) and also necrotic and apoptotic cells (14–15). Phagocytosis of the latter seems to be mediated by CD36, αvβ4 or αvβ5 integrins.

Taken together, these uptake mechanisms allow DC uptake of many types of antigens rapidly, from small solutes by macropinocytosis to large particulate antigens by phagocytosis.

Mature Dendritic Cells

On encounter with "danger signals", immature DC undergo phenotypic changes that results in the transition from an immature to a terminally differentiated mature stage, where the functional properties are shifted from antigen uptake to antigen presentation. Maturation is paralleled by migration of DC through the afferent lymph vessels to the T-cell areas of the secondary lymphoid organs. The maturation process thus continues from the initial encounter with antigen until final T-cell activation in the lymph nodes.

Several danger signals can induce and regulate the maturation process in DC; (i) molecules from pathogens (lipopolysaccharide (6), bacterial DNA (16), double-stranded RNA (17)), (ii) a change in the balance between pro-and anti-inflammatory cytokines in the local environment (tumor necrosis factor (TNF), interleukin (IL) 1, IL-6, IL-10, transforming growth factor (TGF) β) (3,6,18–19), (iii) extracellular matrix degradation products (heparane sulfate (20), hyaluronan oligosaccarides (21), and (iv) T-cell derived signals, e.g., by ligation of CD40.

Multiple cellular events are associated with the DC maturation process. The uptake machinery is down regulated resulting in a low phagocytic activity (6). Molecules needed for migration to the lymph nodes and antigen presentation are upregulated, e.g., the CCR7 chemokine receptor (22) whose ligands are macrophage inflammatory protein 3β and secondary lymphoid-tissue chemokine (22–23).

Efficient antigen presentation requires high levels of MHC complexes and co-stimulatory molecules at the cell surface. DC upregulate co-stimulatory molecules such as CD40, CD58, CD80 and CD86 on maturation (7,24). Both surface MHC Class I and Class II are upregulated.

Finally, maturation is associated with a morphologic change, where the cells lose adherence and alter the shape by cytoskeleton rearrangement (25). Immature DC possess numerous motile, thin cytoplasmic processes or dendrites providing for a large surface area for uptake and stirring of the

surrounding water layer. On maturation these thin processes become larger cytoplasmic veils that are continuously extended and retracted. Prolongation of the dendrites optimizes the surface area for the simultaneous interaction with multiple T-cells.

The life of the DC is ended in the lymph nodes where it is claimed that the cells die shortly after antigen presentation. Figure 1 summarizes the characteristics of immature and mature DC.

Antigen Processing, Presentation, and T-Cell Activation

Antigenic material taken up by DC is degraded intracellularly into peptide fragments that are loaded on MHC molecules and presented to T-cells in the lymph nodes.

Soluble and particulate antigens are after uptake directed to MHC class II compartments (MIIC) in the DC (7,26). The antigens are degraded into peptide fragments due to the lysosomal-like environment in the MIICs that causes weak proteolytic degradation. In immature DC, MHC class II is continuously synthesized and has a fast turnover rate in MIICs (27). On maturation the turnover decreases, there is a burst in MHC class II synthesis, peptide fragments are loaded on the molecules after removal of invariant chain and the complexes are translocated to the cell surface. The decreased turnover rate of MHC antigens on DC maturation leads to an increased duration of the surface peptide presentation, enhancing the probability of encounter with and activation of specific T-cells (28). Meanwhile, the cells migrate to the secondary lymphoid organs where the encounter with MHC class II restricted specific $CD4^+$ T-cells takes place.

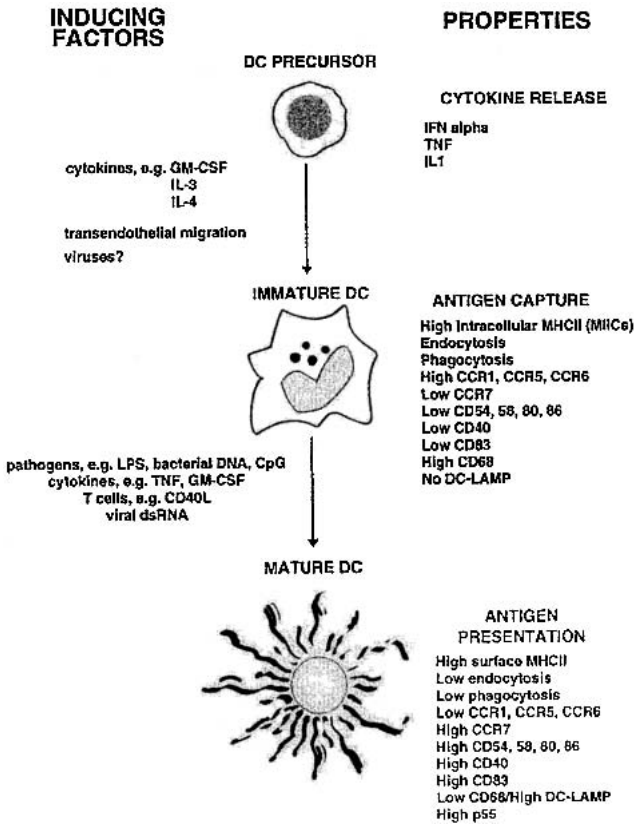

Fig. 1. Maturation of dendritic cells (DC). The left side of the scheme shows the factors inducing progression from one stage to another (GM-CSF, granulocyte/macrophage colony-stimulating factor; IL, interleukin; LPS, lipopolysaccaride; TNF, tumor necrosis factor; dsRNA, double-stranded RNA); the right side shows the main properties of each differentiation/maturation stage (IFN, interferon; MHCII, major histocompatibility complex II; MIIC, MHCII-rich compartment; LAMP, lysosome-associated membrane protein). Reprinted with permission from the Annual Review of Immunology, Volume 18 © 2000 by Annual Reviews www.AnnualReviews.org (3).

Both endogenous and exogenous antigens can be presented on MHC class I molecules that can activate $CD8^+$ CTL (29). Until recently only endogenous proteins were thought to be presented on MHC class I. Cytosolic proteins are degraded into peptide fragments by the proteasome, and peptides are transported into the endoplasmatic reticulum (ER) by molecules called Transporters associated with Antigen Processing (TAP) -1 and -2 in the ER membrane. Peptides are loaded on class I in the ER and complexes are translocated to the cell surface for presentation to specific $CD8^+$ T-cells.

Different mechanisms are thought to be involved in the processing of exogenous antigens presented by MHC class I, a process called cross priming. This mechanism is mainly a feature of DC, but macrophages are to a lesser extent also able to present antigens by cross priming (30). A TAP-dependent and a TAP-independent pathway have been identified. Antigens are selectively transported from what is believed to be a specialized type of endosome to the cytosol by the TAP-dependent pathway (1). A TAP-independent pathway has also been discovered where antigen probably is hydrolyzed in endosomes and peptides are loaded directly onto MHC class I molecules (31). Cross priming seems to be important for certain types of antigens, such as transplantation antigens, particulate antigens, tumor antigens, and viral antigens and in the development of tolerance.

T-cell priming goes on in the T-cell areas of the secondary lymphoid organs (3). DC and T-cell clustering in lymph nodes is mediated by adhesion molecules such as integrinβ1, CD2, CD50, CD54, and CD58. Antigen-specific interaction occurs between peptide-loaded MHC complexes on DC and antigen-specific TCR on T-cells. The second activation signal is mediated by co-stimulatory molecules on DC (CD40, CD80, CD86) and their receptors on T-cells thus sustaining and amplifying the activation. Naïve $CD4^+$ T-cells are primed by soluble antigen-pulsed DC and these can interact with B-cells and stimulate antigen specific antibody production. DC can also prime naïve $CD8^+$ T-cells in the absence of $CD4^+$ T-cells but some antigens have requirement for $CD4^+$ T cell help. DC can also directly activate naïve and memory B-cells to become plasma cells and IgG secreting cells.

An important issue when discussing DC in combination with vaccine design is the identification of the role of DC in inducing tolerance and autoimmunity. Sallusto and Lanzavecchia (32) suggested two possible mechanisms for the role of DC in inducing tolerance. Tolerogenic DC represents a specialized lineage, namely the lymphoid DC, which are derived from a lymphoid progenitor. The lack of IL2 production by this DC subset abolishes the activation of T-cells and induces anergy. The second possible mechanism is that the same type of DC is responsible both for inducing either tolerance or an immune response. The DC maturation stage and the local cytokine environment would determine the outcome of specific T-cell antigen presentation. In this context, a high activation state induces priming of specific T-cells whereas a low activation state causes tolerance or ignorance. For vaccina-

tion purposes the investigation of these different possibilities will be crucial since vaccination, which aim at targeting DC will not only be dependent on the nature of the antigen, dose, drug delivery system and delivery route, but also harbor a risk of inducing antigen specific tolerance.

DC Hematopoiesis

A high degree of heterogeneity exists among DC subsets possibly reflecting the multiple functions of this cell type (reviewed in (33) and (34)). There is evidence for the existence of at least two different human $CD34^+$ hematopoietic precursors, one of the myeloid lineage, that gives rise to granulocytes/monocytes and myeloid DC, and one of the lymphoid lineage giving rise to T-, B-, NK-cells and lymphoid DC. DC lineage relationships are shown in Fig. 2.

$CD11^+$ $CD14^+$ monocytes differentiate into immature interstitial DC in the presence of granulocyte macrophage colony stimulating factor (GM-CSF) and IL-4 (7). Myeloid progenitors can moreover differentiate into $CD14^-$ $CD11c^+$ precursors that develop into LC in the presence of GM-CSF, IL-4 and TGFβ (36). Both types of precursors can also differentiate into macrophages in response to macrophage colony stimulating factor showing the close relationship between DC and macrophages. Functional differences between these two DC subsets exist. Only interstitial DC but not LC can induce differentiation of naïve B-cells *in vitro* (37). Moreover, interstitial DC express IL-10 and have a 10-fold higher antigen capture efficiency than LC. LC have been suggested to be involved in the priming of $CD8^+$ T-cells (3).

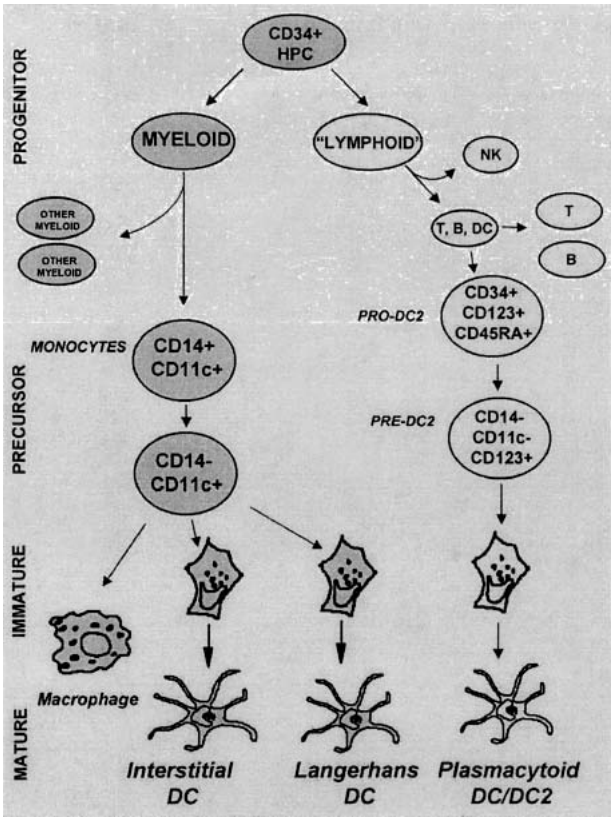

Fig. 2. Potential differentiation pathways of human DC. Myeloid $CD34^+$ progenitors differentiate into monocytes ($CD14^+$ $CD11c^+$ DC precursors) that yield circulating $CD11c^+$ precursors. $CD11c^+$ cells can differentiate into either macrophages or interstitial DC or LC depending on the cytokine microenvironment. A distinct precursor population may give rise to plasmacytoid DC. In some circumstances, this population also contains cells that can yield lymphocytes, hence the current term 'lymphoid'. $CD34^+$ cells contain a subset with the phenotypic and functional characteristics of DC2, pro-DC2, with a limited proliferative capacity. Thus, human blood contains two DC precursors, $CD11c^+$ (myeloid) and $CD11c^-$ (potentially lymphoid). Reproduced from the Journal of Experimental Medicine, 2000, Volume 192, Number 12, pp. 741 by copyright permission of the Rockefeller University Press (35).

The existence of a common human lymphoid precursor is still controversial, but a $CD14^-$ $CD11c^+$ IL-3Rα$^+$ precursor may originate from $CD34^+$ lymphoid hematopoietic progenitors (reviewed in (34)). These precursors can differentiate into lymphoid DC in the presence of IL-3 (38).

In summary, DC constitute a heterogeneous cell type with subtypes in many different tissues. The complexity of DC increases by the existence of several developmental stages with specialized functions in physically different places. The heterogeneity of DC subsets should be understood in the light of the different functions and the multiple anatomic localizations. Each subset seems to be specialized to perform specific tasks, but lineage origins and functional differences are not fully clarified yet. A better understanding of DC multiple roles in inducing immunity, tolerance, and autoimmunity will be a prerequisite for the design of efficient and safe vaccine formulations.

OPTIMIZING IMMUNIZATIONS BY TARGETING TO DC

In light of the described functional characteristics of DC, an obvious strategy for designing new or better vaccine formulations is a more defined targeting of antigens to this cell type thereby exploiting the unique abilities of DC for antigen acquisition and display. A pre-requisite for vaccines to be effective is that antigens are acquired and displayed by APC. *Ex vivo* peptide/protein-pulsed or gene-modified DC have been used in experimental model systems of cancer and are shown to induce strong $CD8^+$ CTL mediated antitumor responses. This "vaccination" approach is highly experimental, expensive and elaborate, and is not suited for larger, human immunization programs, but illustrates well the pronounced effect of targeted antigen delivery into DC. *In vivo* targeting of vaccines to DC is highly desirable, and optimizing delivery systems with this aim in mind is expected to improve efficacy, reduce doses and the risk of side effects, and improve control of immunologic outcome. The last sections will describe possible ways to target DC or to modulate the immune response through the action of DC.

DC Surface Receptor Targeting

Several studies point to the fact that targeting antigens to receptors on DC can be expected to enhance immune responses. FcγR I and II, that bind the Fc-part of IgG, mediate internalization of IC thereby sensitizing DC for priming of both $CD4^+$ helper cells and $CD8^+$ CTL *in vivo* (7,39). You *et al.* (40) immunized mice intramuscularly with a DNA vaccine encoding a fusion between model hepatitis B virus e antigen and an IgG F_c fragment. The fusion protein is thus expressed after vaccination and can be captured and processed by DC, probably via FcγR. A more efficient induction of antigen

specific $CD4^+$ Th1 cells, $CD8^+$ CTL and B-cell responses by fusion of antigen to the F_c part if IgG than with antigen alone was observed. Bot *et al.* (41) tested spray-dried lipid-based microparticles containing inactivated influenza virus with or without IgG coupled in rodents. Microparticles without IgG targeting elicited lower responses than administration of plain, inactivated virus. The level of T-cell response was restored both *in vitro* and *in vivo* by incorporating IgG in the microparticles. Wallace *et al.* (42) tested in a human myeloid cell line THP-1 a DNA construct encoding a fusion of the Fab region of a monoclonal antibody directed against FcγRI and the tumor protein prostate specific antigen. This very specific targeting of FcγRI avoids unspecific binding to other classes of FcγR that also exists in many cell types that are not efficient APCs. The fusion protein could be taken up in an FcγRI specific manner, processed, and prostate specific antigen peptides were presented in an MHC class I restricted manner. Prostate specific antigen-specific human CTL were able to lyse THP1 and pretreatment with blocking agents blocked lysis. It was concluded that uptake via FcγRI thus results in cross priming.

In general, IC are internalized with the FcγR into DC, and receptor-ligand complexes are degraded which results in a single round of uptake. This non-recycling type of receptor has also a signaling function (FcγRI) in DC by inducing maturation (39). MHC class II restricted antigen presentation is more than 100-fold more efficient when mediated by FcγRII than by fluid phase pinocytosis *in vitro* (7). Specific for DC is that FcγR also promotes efficient MHC class I-restricted presentation that is dependent on proteasomal degradation and TAP (39). After internalization, antigens for class I presentation transfer to the cytosol and enter the class I presentation pathway. Targeting antigens to FcγR, despite their lower uptake capacity than MMR and DEC-205 (see later), is expected to be advantageous for antigens that should reach both MHC class I and class II presentation pathways simultaneously. $CD4^+$ T-cell help is namely required for efficient $CD8^+$ T cell priming that happens by antigen recognition by both $CD4^+$ and $CD8^+$ T-cells on the same DC. FcγR thus represent a connection between humoral and cytotoxic components of immune responses and are potential targets for vaccination strategies against e.g., intracellular microorganisms and tumors.

Another receptor on DC targeting the MHC class I processing pathway is the glycolipid globotriacylceramid (Gb3) (43). Gb3 is a neutral glycosphingolipid present on the DC cell surface and on some human epithelial and endothelial cells. The presence of Gb3 on DC was noted in a single study where intraperitoneal immunization of mice with non-toxic Shiga toxin B fused to the tumor antigen P815A induced CTL responses in the absence of adjuvants via a DC surface receptor-dependent process involving Gb3. Little is known about Gb3 in DC, but it represents a putative, alternative way of targeting antigen-Shiga B toxin fusions or yet unidentified ligands that stimulate class I immune responses.

Only few studies have been performed so far that exploit targeting of MMR. Diebold *et al.* (44) synthesized delivery systems for DNA composed of mannosylpolyethylenimine conjugates and showed that uptake into MMR-expressing cells was MMR-specific. Moreover, mannosylated polyethyleneimine was more potent in delivering a luciferace reporter gene into DC than unconjugated polyethylenimine, although transfection efficiency was low. Inclusion of adenovirus in the delivery system enhanced the transgene expression, probably by facilitating DNA release from the endosomal compartment.

The MMR has multiple carbohydrate binding domains that provide broad substrate specificity toward mannosylated/fucosylated entities. Mannosylated proteins are abundant in many microorganisms, as opposed to eukaryots, and these are therefore "naturally targeted" to DC. Sugar-containing substrates are taken up by the MMR into vesicular structures different from the MIIC, where the cargo is released, and MMR is transported back to the cell surface (11). The MMR is thereby constitutively recycled between the endosomes and the plasma membrane resulting in a sustained capacity for antigen capture concentrating large amounts of antigens intracellularly. The receptor is involved in antigen uptake only and not presentation since free antigens only after release are transported to MIIC and delivered to the MHC class II presentation pathway. Uptake of glycosylated antigen via the MMR can enhance 100-10.000 fold the presentation of soluble antigen *in vitro* (11,45). MMR-targeting is thus expected to be advantageous in eliciting protective immunity for MHC class II-restricted antigens.

However, some antigens targeting the MMR fail to elicit immune responses. Hiltbold *et al.* (46) showed that the tumor glycoprotein MUC1 does not stimulate strong immune responses even though the antigen is taken up by DC via MMR. This is explained by high-avidity binding between MMR and large number of sugar residues along the protein backbone, which prevent dissociation in the early endosomes where MMR-MUC1 complexes are trapped. In conclusion, MMR-targeting does so far not appear to be beneficial and further studies are needed to conclude whether targeting to MMR has a potential for vaccine targeting or not.

DEC-205 is expected to have a similar or even larger potential in targeting as MMR, since this receptor is specific for DC. Mancke *et al.* (47) showed that DEC-205 is far more superior to MMR in presenting peptides to T-cells when using polyclonal rabbit antibodies against DEC-205 as surrogate ligands. DEC-205-mediated uptake enhance 100-fold the MHC class II presentation of soluble antigen but by a mechanism different from MMR, since uptake targets late endosomes or lysosomes rich in MHC class II molecules (47). Recycling of free receptor to the cell surface equip DEC-205 with the same, high antigen capturing potential as MMR. A prerequisite for further exploiting the possibility of using DEC-205 for targeting MHC class II presentation is however the identification of ligands for the receptor.

Attempts have been made to target B7 molecules specifically expressed on APC with cytotoxic T-lymphocyte antigen-4 (CTLA-4) antigen fusions. Such approach is not specific for DC but will target APC in general. Deliyannis *et al.* (48) showed that a fusion of hemagglutinin-based influenza virus DNA and CTLA-4 in a DNA vaccine accelerated and increased antibody response compared to a non-targeted control on intramuscular immunization in mice. CTL responses were not enhanced, so the observed 100-fold reduced viral titers in mice vaccinated with the CTLA-4 fusion after a nonlethal virus challenge was explained by enhanced antibody response. Chaplin *et al.* (49) immunized sheeps with a DNA vaccine encoding detoxified phospholipase D fused to the CTLA-4 gene. Detoxified phospholipase D is partially effec-

tive against *Corynebacterium pseudotuberculosis* in sheep. Targeting to CTLA-4 enhanced speed, magnitude and longevity of the antibody response when compared to the non-targeted DNA vaccine and afforded better protection against a non-lethal challenge with *C. pseudotuberculosis*.

Finally, Drew *et al.* (50) immunized mice intramuscularly with a DNA vaccine encoding a fusion between CTLA-4, human immunoglobulin and the host-protective 45W antigen from *Taena ovis*. A 30-fold higher 45W specific antibody response was noted in mice immunized with the CTLA-4 targeted DNA vaccine compared to the non-targeted control. Moreover, faster kinetics was observed since the development of antibody production was accelerated. The same construct failed to enhance immune responses in sheep.

In all cases the APC targeting enhances the immune response induced by DNA vaccines in an antibody dependent way. The mechanism of immune response enhancement is not antigen through antigen endocytosis mediated by B7, but more likely an increase in binding of antigen to the DC surfaces thereby enhancing antigen capture. Targeting B7 is thus advantageous for DNA vaccines that do not induce sufficient antibody responses.

Several other molecules have been investigated to target APC in general. Hung *et al.* (51) tested a DNA vaccine targeting Flt3 with Flt3-ligand. Flt-3 expression is, in hematopoietic tissue, restricted to $CD34^+$ progenitors, including DC progenitors, and fusing antigens to Flt3-ligand is thus expected also to target DC. Human papillomavirus 16 E7 was used as model antigen and fused to the extracellular domain of Flt3-ligand in a DNA construct and administered intradermally via a gene gun to mice. The fusion gene increased significantly the frequency of E7-specific $CD8^+$ T-cells but not $CD4^+$ T-cell compared to a non-fused control DNA vaccine. The targeted vaccine, compared to the control, was able to control lethal E7-expressing metastatic tumors in mice.

Finally, Tillman *et al.* (52–53) explored the potential of targeting adenoviral vectors to CD40 that is expressed at high levels on DC. Adenovirus targeted to CD40 via a bi-specific antibody enhances gene transfer to DC compared to an untargeted adenovirus vector. The vector itself is able to induce maturation due to the CD40 binding and is thus multifunctional by both targeting and activating DC. In a murine model, DC infected with adenovirus encoding the human papillomavirus E7 antigen targeted to CD40 enhanced protection against human papillomavirus-induced tumor cells.

Which uptake mechanism should we then target to get most efficient antigen presentation? That depends clearly on the type of immune response needed to provoke clearance of disease. The type of receptor influences whether MHC class I and/or MHC class II presentation occurs. In this respect targeting immune cell compartments may be a just as important approach as targeting on the receptor level. Targeting the class I pathway has attracted much interest, and development of methods for delivery of exogenous antigen into this pathway has focused on coupling antigen to cationic or fusogenic peptides to facilitate uptake and on improving access from the endosomal route into the cytosol. Alternatively, endogenous delivery into the class I presentation pathway of DC can be achieved by viral vectors, mRNA (54), DNA vaccines transfecting DC directly or by transfection of other cell types with the antigen and by a death signal inducing apoptosis. Apoptotic cells are phagocytosed by DC and presented in a class I-restricted manner (14). Cell death might therefore be important for transfer of antigen from non-professional to professional APC. Furthermore, engineered Fas-mediated apoptotic death of antigen bearing cells (transfected) increases antigen acquisition by APC (including DC), enhances antigen-specific CTL and induces Th1 type cytokines in mice (55). It is believed that danger signals in this situation are important for immune stimulation: Presentation of antigens from apoptotic cells by DC in lymph nodes in the absence of maturation signals (steady state) is suggested to be a mechanism for induction of peripheral tolerance (26). Further exploration of this powerful targeting principle for vaccination purposes is awaited with great interest.

Implementing DC targeting into a developable vaccine formulation requires that the targeting principle is simple, well understood and easily applicable without for example expensive and elaborate conjugation procedures. Several strategies can be followed to incorporate a targeting feature into vaccine formulations; 1) at protein/peptide level, 2) at DNA level (DNA vaccine) with endogenous synthesis of antigen or synthesis of chimeric protein targeted to DC or 3) at the level of the drug delivery system. DNA vaccines seem to incorporate the targeting principle most easily and targeting might well contribute to the highly demanded enhancement of DNA vaccine efficiency in humans.

Alerting DC for Action

The chosen DDS for a vaccine could in addition to just passive protection and active targeting possess additional features that modulate or activate DC. The growth factors GM-CSF, Flt3 ligand and G-CSF have been shown to act as mobilisators/recruitment factors for DC. Treatment of humans with these cytokines mobilizes DC precursors from the bone marrow into the blood. Increasing the number of DC by Flt3 ligand can enhance the immune response to vaccine antigens in mice (56). Co-administration of this type of growth factors with antigens in vaccine formulations is therefore expected to have a great potential by increasing the size of the DC population.

The vaccine DDS could also present a signal that stimulates activation, maturation and migration of DC. Many substances are known to act in these processes. Among these are traditional adjuvants like Freunds incomplete adjuvant, LPS, CpG dinucleotides, CD40 ligand and cytokines like TNFα, IL10, IL4, IL12, IL-1β and Interferon γ. The efficiency of these adjuvants within a vaccine formulation is dependent on the type of pathogen and the type of immune response that is needed to clear the infection.

Focus has recently been on the role of DC in the regulation of immune responses (see (57) for review). DC function seems to be determined by many different factors that govern the final outcome of immune response, that would say Th1 vs. Th2 response. Moreover, different cytokines mobilize distinct DC subsets and different DC subsets appear to induce different immune responses. Ideally for vaccinology purposes, one should know which subsets induce which responses and which cytokines mobilize which subsets. The area is very complex and review of the literature is beyond the scope of this review, but in brief Pulendran *et al.* (57) have proposed the following model: The final Th-cell polarization is determined by 1) the microbial product/adjuvant, 2) the receptors on DC through

which the adjuvant signals, 3) the DC subset itself, 4) the local microenvironment and 5) the cytokines released by neighboring T-cells and other cells. The hypothesis is therefore that the function of DC is not fixed but is adaptable in response to signals from the microenvironment and the pathogen. By co-delivering immunomodulators or immunopotentiators which mechanism of action is well known the outcome of the immune response can hopefully be directed more precisely than with traditional adjuvants.

Vaccine Drug Delivery Systems

The eradication of diseases like small pox by vaccination shows that large global immunization programs can be very effective even without any targeting to specific cells of the immune system. Live or killed vaccines has been found to evolutionarily be targeted and taken up by DC e.g., via the MMR or FcγR. However, in the design of DDS for new vaccines, investigating and improving the targeting properties to DC may appear to give useful information and enhance efficacy.

Most pharmaceutical antigens are macromolecules, either peptides/proteins or DNA molecules. Therefore they exert the same weaknesses as other proteins and macromolecular drug candidates in terms of stability and bioavailability. Particulate delivery of macromolecules seems to overcome these weaknesses and many different systems are tested. These include polymeric materials ranging from poly-lactide-co-glycolide, starch and a large number of other natural and synthetic polymers, and also lipid systems such as microemulsions and liposomes. Commonly investigated vaccine DDS are shown in Table I and will not be discussed further (2,58).

Particulate systems are naturally targeted to APC since their dimensions are comparable to those of microorganisms. In addition, particles can through phagocytosis efficiently deliver antigen to APC 1000–10.000 fold more efficient than soluble antigen and mediate the induction of both MHC class I and class II responses in DC. Particles are thus multifunctional in that they deliver antigens to DC in a form where antigen is concentrated in spherical structures and protected against premature degradation. Moreover they may act as adjuvants that are able to mediate both types of immune responses (59).

A huge challenge lies in the requirements for particle delivery technology. Pharmaceutically acceptable particulate delivery systems should be developed that are safe, well tolerated, easy to administer, easy to store and inexpensive.

Particulate systems in themselves are able to mediate sufficient immune responses when delivered at sites with high APC density because they have intrinsic ability to target APCs. Further targeting to DC might appear to be necessary for DNA vaccines that have provoked very weak immune responses in humans. Targeting to a surface receptor on DC might enhance the specific immune response to a degree that confers protection even in larger animals and humans.

Finally microparticles have the potential to incorporate well-defined adjuvants such as cytokines to further enhance or modulate immune responses to the desired type.

Big challenges for the formulation scientist lie in the requirements for particle delivery technology. Pharmaceutically acceptable particulate delivery systems that are safe, well tolerated, easy to administer, easy to store and inexpensive need to be developed.

Table I. Vaccine Drug Delivery Systems

Lipid particles:
Oil emulsions (e.g. Freunds Incomplete and Complete)
ISCOMS
Liposomes
Biodegradable polymeric microparticles
PLG, starch, gelatin, chitosan, alginate, dextran
Live recombinant vectors—traditional
Virus-like particles

Route of Administration

How to administer a vaccine to get a good immune response might be an even more important question than the previously mentioned targeting strategies. Bringing sufficient amount of vaccine encapsulated in a particulate system in close proximity to DC might be sufficient to obtain an immune response.

Vaccines may be administered either parenterally or mucosally. Parenteral immunization elicits only systemic immune responses and no mucosal protection and may therefore be well suited for immunization against a disease like cancer. The fate of the parenteral vaccine is very much dependent on the immunologic competence of the cells at the injection site. The skin has a large immunologic potential since LC are found in the viable epidermis, where they comprise 1% of the cell population (60). Despite their low percentage, LC have a large spatial extent comprising 20% of the surface area. On damage of the skin, keratinocytes can synthesize cytokines involved in modulating the immune response. Thereby LC receive activation signals and leave the skin bringing the putatively invasive antigens to the regional lymph nodes for induction of immune responses. The skin thus has a pronounced immunologic potential for initiating primary immune responses on delivery of vaccines. Depots of antigens in the skin do not only stimulate immune responses, but do also act as adjuvants by stimulating DC to increase acquisition of antigen. Delivering antigens to the skin appears very promising, and developing DDS more effective that commonly used gene gun (DNA) or needle-free jet injection devices are interesting alternatives, that could be patches, creams or gels for topical administration.

For parenteral administration, future effective vaccines may solely be dependent on targeting DC, either by delivering antigens as particles in close proximity to DC or by adding a targeting moiety.

The integrated mucosal immune system protects against pathogens that invade through the mucosa and mediates tolerance against bacterial flora and soluble antigens. Mucosal immunization is important for protection against pathogens that infect via the mucosal route (oral, nasal, pulmonal, rectal, vaginal) as for example influenza and HIV and offers the advantage that both mucosal and systemic immunity is obtained, but harbor also the risk of inducing tolerance. Larger particulate antigens like virus and bacteria are thought to be taken up via M-cells into mucosa-associated lymphoid tissue (61). M-cells are highly phagocytotic epithelial cells that on attachment of particulate antigens and macromolecules translocate them to underlying mucosa-associated lymphoid tissue

where DC are present in large numbers. In the gut, these lymphoid structures lined by M-cells are called Peyers patches. The M-cells of Peyers patches lack brush border microvilli, thereby facilitating entrance, but they only constitute a minor part of the mucosal surface. For mucosal vaccination, uptake into M-cells and not the delivery to DC might be the efficient rate-limiting step. Targeting M-cells instead is therefore expected to have much higher impact than targeting DC. Therefore, DDS for mucosal delivery as opposed to parenteral delivery should probably target M-cells and protect the antigen against degradation in the mucosal environment and not focus much on the ability to target DC.

Depending on the aim of the formulation project the formulation scientist must evaluate very closely a number of parameters as depicted in Table II. The DDS chosen for each vaccine formulation must thus be selected on a rational basis of many parameters. One example is the development of a cancer vaccine. Because particles stimulate both class I and II responses via DC, the particulate formulation strategy is interesting in the context of a cancer vaccine. Moreover it would be of great benefit if a cell-specific targeting could be attained to increase or redirect the immune response, especially in the case of DNA vaccines. In this case it may not be worthwhile to aim for mucosal delivery, thus e.g. subcutaneous administration may be sufficient.

CONCLUSIONS AND PERSPECTIVES

There is a great potential hidden in optimizing antigen delivery to DC by single approaches or by combining multiple approaches at different levels such as receptor targeting, DDS, immunomodulators and administration route. For each pathogen we needs to understand the necessary and sufficient immune response required to protect while minimizing pathology. This knowledge can help us designing vaccine formulations that target the right DC subset and that fully stimulate the necessary arms of the immune system and thereby providing us with a tool to control more precisely the immunologic outcome. Much work has to be done to understand which of the approaches will be most useful for human immunization.

Table II. Parameters Important for Formulation of Vaccines

Nature of antigen:
Chemical nature (proteinacious or DNA)
Size
Stability
Biopharmaceutical properties (solubility, P-chem.)
Nature of pathogen or disease:
Viral/bacterial/other
Route of entry
Nature of origin
Progression of disease
Desired immune response:
Humoral
CTL
Th1/Th2
Epidemiology:
Requirements for DDS:
Administration route
Shelf life
Targeting
Delivery device

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REFERENCES

1. A. Rodriguez, A. Regnault, M. Kleijmeer, P. Ricciardi-Castagnoli, and S. Amigorena. Selective transport of internalized antigens to the cytosol for MHC class I presentation in dendritic cells. *Nat. Cell Biol.* **1**:362–368 (1999).
2. M. Singh and D. O'Hagan. Advances in vaccine adjuvants. *Nat. Biotechnol.* **17**:1075–1081 (1999).
3. J. Banchereau, F. Briere, C. Caux, J. Davoust, S. Lebecque, Y. J. Liu, B. Pulendran, and K. Palucka. Immunobiology of dendritic cells. *Annu. Rev. Immunol.* **18**:767–811 (2000).
4. A. Tang, M. Amagai, L. G. Granger, J. R. Stanley, and M. C. Udey. Adhesion of epidermal Langerhans cells to keratinocytes mediated by E- cadherin. *Nature* **361**:82–85 (1993).
5. D. N. Hart. Dendritic cells: unique leukocyte populations which control the primary immune response. *Blood* **90**:3245–3287 (1997).
6. F. Sallusto, M. Cella, C. Danieli, and A. Lanzavecchia. Dendritic cells use macropinocytosis and the mannose receptor to concentrate macromolecules in the major histocompatibility complex class II compartment: downregulation by cytokines and bacterial products. *J. Exp. Med.* **182**:389–400 (1995).
7. F. Sallusto and A. Lanzavecchia. Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor alpha. *J. Exp. Med.* **179**:1109–1118 (1994).
8. R. M. Steinman and J. Swanson. The endocytic activity of dendritic cells. *J. Exp. Med.* **182**:283–288 (1995).
9. A. de Baey and A. Lanzavecchia. The role of aquaporins in dendritic cell macropinocytosis. *J. Exp. Med.* **191**:743–748 (2000).
10. W. Jiang, W. J. Swiggard, C. Heufler, M. Peng, A. Mirza, R. M. Steinman, and M. C. Nussenzweig. The receptor DEC-205 expressed by dendritic cells and thymic epithelial cells is involved in antigen processing. *Nature* **375**:151–155 (1995).
11. M. C. Tan, A. M. Mommaas, J. W. Drijfhout, R. Jordens, J. J. Onderwater, D. Verwoerd, A. A. Mulder, A. N. van der Heiden, D. Scheidegger, L. C. Oomen, T. H. Ottenhoff, A. Tulp, J. J. Neefjes, and F. Koning. Mannose receptor-mediated uptake of antigens strongly enhances HLA class II-restricted antigen presentation by cultured dendritic cells. *Eur. J. Immunol.* **27**:2426–2435 (1997).
12. K. Inaba, M. Inaba, M. Naito, and R. M. Steinman. Dendritic cell progenitors phagocytose particulates, including bacillus Calmette-Guerin organisms, and sensitize mice to mycobacterial antigens in vivo. *J. Exp. Med.* **178**:479–488 (1993).
13. C. Scheicher, M. Mehlig, H. P. Dienes, and K. Reske. Uptake of microparticle-adsorbed protein antigen by bone marrow-derived dendritic cells results in up-regulation of interleukin-1 alpha and interleukin-12 p40/p35 and triggers prolonged, efficient antigen presentation. *Eur. J. Immunol.* **25**:1566–1572 (1995).
14. M. L. Albert, S. F. Pearce, L. M. Francisco, B. Sauter, P. Roy, R. L. Silverstein, and N. Bhardwaj. Immature dendritic cells phagocytose apoptotic cells via alphavbeta5 and CD36, and cross-present antigens to cytotoxic T lymphocytes. *J. Exp. Med.* **188**:1359–1368 (1998).
15. B. Sauter, M. L. Albert, L. Francisco, M. Larsson, S. Somersan, and N. Bhardwaj. Consequences of cell death: exposure to necrotic tumor cells, but not primary tissue cells or apoptotic cells, induces the maturation of immunostimulatory dendritic cells. *J. Exp. Med.* **191**:423–434 (2000).
16. O. Akbari, N. Panjwani, S. Garcia, R. Tascon, D. Lowrie, and B. Stockinger. DNA vaccination: transfection and activation of den-

dritic cells as key events for immunity. *J. Exp. Med.* **189**:169–178 (1999).
17. M. Cella, M. Salio, Y. Sakakibara, H. Langen, I. Julkunen, and A. Lanzavecchia. Maturation, activation, and protection of dendritic cells induced by double-stranded RNA. *J. Exp. Med.* **189**:821–829 (1999).
18. T. De Smedt, M. Van Mechelen, G. De Becker, J. Urbain, O. Leo, and M. Moser. Effect of interleukin-10 on dendritic cell maturation and function. *Eur. J. Immunol.* **27**:1229–1235 (1997).
19. F. Geissmann, P. Revy, A. Regnault, Y. Lepelletier, M. Dy, N. Brousse, S. Amigorena, O. Hermine, and A. Durandy. TGF-beta 1 prevents the noncognate maturation of human dendritic Langerhans cells. *J. Immunol.* **162**:4567–4575 (1999).
20. Y. Kodaira, S. K. Nair, L. E. Wrenshall, E. Gilboa, and J. L. Platt. Phenotypic and functional maturation of dendritic cells mediated by heparan sulfate. *J. Immunol.* **165**:1599–1604 (2000).
21. C. C. Termeer, J. Hennies, U. Voith, T. Ahrens, J. M. Weiss, P. Prehm, and J. C. Simon. Oligosaccharides of hyaluronan are potent activators of dendritic cells. *J. Immunol.* **165**:1863–1870 (2000).
22. M. C. Dieu, B. Vanbervliet, A. Vicari, J. M. Bridon, E. Oldham, S. Ait-Yahia, F. Briere, A. Zlotnik, S. Lebecque, and C. Caux. Selective recruitment of immature and mature dendritic cells by distinct chemokines expressed in different anatomic sites. *J. Exp. Med.* **188**:373–386 (1998).
23. V. W. Chan, S. Kothakota, M. C. Rohan, L. Panganiban-Lustan, J. P. Gardner, M. S. Wachowicz, J. A. Winter, and L. T. Williams. Secondary lymphoid-tissue chemokine (SLC) is chemotactic for mature dendritic cells. *Blood* **93**:3610–3616 (1999).
24. N. Romani, D. Reider, M. Heuer, S. Ebner, E. Kampgen, B. Eibl, D. Niederwieser, and G. Schuler. Generation of mature dendritic cells from human blood. An improved method with special regard to clinical applicability. *J. Immunol. Methods* **196**:137–151 (1996).
25. C. Winzler, P. Rovere, M. Rescigno, F. Granucci, G. Penna, L. Adorini, V. S. Zimmermann, J. Davoust, and P. Ricciardi-Castagnoli. Maturation stages of mouse dendritic cells in growth factor-dependent long-term cultures. *J. Exp. Med* **185**:317–328 (1997).
26. K. Inaba, S. Turley, F. Yamaide, T. Iyoda, K. Mahnke, M. Inaba, M. Pack, M. Subklewe, B. Sauter, D. Sheff, M. Albert, N. Bhardwaj, I. Mellman, and R. M. Steinman. Efficient presentation of phagocytosed cellular fragments on the major histocompatibility complex class II products of dendritic cells. *J. Exp. Med.* **188**: 2163–2173 (1998).
27. M. J. Kleijmeer, M. A. Ossevoort, C. J. van Veen, J. J. van Hellemond, J. J. Neefjes, W. M. Kast, C. J. Melief, and H. J. Geuze. MHC class II compartments and the kinetics of antigen presentation in activated mouse spleen dendritic cells. *J. Immunol.* **154**: 5715–5724 (1995).
28. R. M. Steinman, K. Inaba, S. Turley, P. Pierre, and I. Mellman. Antigen capture, processing, and presentation by dendritic cells: recent cell biologic studies. *Hum. Immunol.* **60**:562–567 (1999).
29. C. C. Norbury, B. J. Chambers, A. R. Prescott, H. G. Ljunggren, and C. Watts. Constitutive macropinocytosis allows TAP-dependent major histocompatibility complex class I presentation of exogenous soluble antigen by bone marrow-derived dendritic cells. *Eur. J. Immunol.* **27**:280–288 (1997).
30. M. Kovacsovics-Bankowski, K. Clark, B. Benacerraf, and K. L. Rock. Efficient major histocompatibility complex class I presentation of exogenous antigen on phagocytosis by macrophages. *Proc. Natl. Acad. Sci. USA* **90**:4942–4946 (1993).
31. J. D. Pfeifer, M. J. Wick, R. L. Roberts, K. Findlay, S. J. Normark, and C. V. Harding. Phagocytic processing of bacterial antigens for class I MHC presentation to T cells. *Nature* **361**:359–362 (1993).
32. F. Sallusto and A. Lanzavecchia. Mobilizing dendritic cells for tolerance, priming, and chronic inflammation. *J. Exp. Med.* **189**: 611–614 (1999).
33. M. Cella, F. Sallusto, and A. Lanzavecchia. Origin, maturation and antigen presenting function of dendritic cells. *Curr. Opin. Immunol.* **9**:10–16 (1997).
34. M. Nouri-Shirazi, J. Banchereau, J. Fay, and K. Palucka. Dendritic cell based tumor vaccines. *Immunol. Lett.* **74**:5–10 (2000).
35. J. Banchereau, B. Pulendran, R. Steinman, and K. Palucka. Will the making of plasmacytoid dendritic cells in vitro help unravel their mysteries? *J. Exp. Med.* **192**:F39–F44 (2000).
36. T. Ito, M. Inaba, K. Inaba, J. Toki, S. Sogo, T. Iguchi, Y. Adachi, K. Yamaguchi, R. Amakawa, J. Valladeau, S. Saeland, S. Fukuhara, and S. Ikehara. A CD1a+/CD11c+ subset of human blood dendritic cells is a direct precursor of Langerhans cells. *J. Immunol.* **163**:1409–1419 (1999).
37. C. Caux, C. Massacrier, B. Vanbervliet, B. Dubois, I. Durand, M. Cella, A. Lanzavecchia, and J. Banchereau. CD34+ hematopoietic progenitors from human cord blood differentiate along two independent dendritic cell pathways in response to granulocyte-macrophage colony-stimulating factor plus tumor necrosis factor alpha: II. Functional analysis. *Blood* **90**:1458–1470 (1997).
38. G. Grouard, M. C. Rissoan, L. Filgueira, I. Durand, J. Banchereau, and Y. J. Liu. The enigmatic plasmacytoid T cells develop into dendritic cells with interleukin (IL)-3 and CD40-ligand. *J. Exp. Med* **185**:1101–1111 (1997).
39. A. Regnault, D. Lankar, V. Lacabanne, A. Rodriguez, C. Thery, M. Rescigno, T. Saito, S. Verbeek, C. Bonnerot, P. Ricciardi-Castagnoli, and S. Amigorena. Fcgamma receptor-mediated induction of dendritic cell maturation and major histocompatibility complex class I-restricted antigen presentation after immune complex internalization. *J. Exp. Med.* **189**:371–380 (1999).
40. Z. You, X. Huang, J. Hester, H. C. Toh, and S. Y. Chen. Targeting dendritic cells to enhance DNA vaccine potency. *Cancer Res.* **61**:3704–3711 (2001).
41. A. I. Bot, D. J. Smith, S. Bot, L. Dellamary, T. E. Tarara, S. Harders, W. Phillips, J. G. Weers, and C. M. Woods. Receptor-mediated targeting of spray-dried lipid particles coformulated with immunoglobulin and loaded with a prototype vaccine. *Pharm. Res.* **18**:971–979 (2001).
42. P. K. Wallace, K. Y. Tsang, J. Goldstein, P. Correale, T. M. Jarry, J. Schlom, P. M. Guyre, M. S. Ernstoff, and M. W. Fanger. Exogenous antigen targeted to FcgammaRI on myeloid cells is presented in association with MHC class I. *J. Immunol. Methods* **248**:183–194 (2001).
43. N. Haicheur, E. Bismuth, S. Bosset, O. Adotevi, G. Warnier, V. Lacabanne, A. Regnault, C. Desaymard, S. Amigorena, P. Ricciardi-Castagnoli, B. Goud, W. H. Fridman, L. Johannes, and E. Tartour. The B subunit of Shiga toxin fused to a tumor antigen elicits CTL and targets dendritic cells to allow MHC class I-restricted presentation of peptides derived from exogenous antigens. *J. Immunol.* **165**:3301–3308 (2000).
44. S. S. Diebold, M. Kursa, E. Wagner, M. Cotten, and M. Zenke. Mannose polyethylenimine conjugates for targeted DNA delivery into dendritic cells. *J. Biol. Chem.* **274**:19087–19094 (1999).
45. A. J. Engering, M. Cella, D. Fluitsma, M. Brockhaus, E. C. Hoefsmit, A. Lanzavecchia, and J. Pieters. The mannose receptor functions as a high capacity and broad specificity antigen receptor in human dendritic cells. *Eur. J. Immunol.* **27**:2417–2425 (1997).
46. E. M. Hiltbold, A. M. Vlad, P. Ciborowski, S. C. Watkins, and O. J. Finn. The mechanism of unresponsiveness to circulating tumor antigen MUC1 is a block in intracellular sorting and processing by dendritic cells. *J. Immunol.* **165**:3730–3741 (2000).
47. K. Mahnke, M. Guo, S. Lee, H. Sepulveda, S. L. Swain, M. Nussenzweig, and R. M. Steinman. 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 (2000).
48. G. Deliyannis, J. S. Boyle, J. L. Brady, L. E. Brown, and A. M. Lew. A fusion DNA vaccine that targets antigen-presenting cells increases protection from viral challenge. *Proc. Natl. Acad. Sci. USA* **97**:6676–6680 (2000).
49. P. J. Chaplin, R. De Rose, J. S. Boyle, P. McWaters, J. Kelly, J. M. Tennent, A. M. Lew, and J. P. Scheerlinck. Targeting improves the efficacy of a DNA vaccine against Corynebacterium pseudotuberculosis in sheep. *Infect. Immun.* **67**:6434–6438 (1999).
50. D. R. Drew, J. S. Boyle, A. M. Lew, M. W. Lightowlers, P. J. Chaplin, and R. A. Strugnell. The comparative efficacy of CTLA-4 and L-selectin targeted DNA vaccines in mice and sheep. *Vaccine* **19**:4417–4428 (2001).
51. C. F. Hung, K. F. Hsu, W. F. Cheng, C. Y. Chai, L. He, M. Ling, and T. C. Wu. Enhancement of DNA vaccine potency by linkage of antigen gene to a gene encoding the extracellular domain of

Fms-like tyrosine kinase 3-ligand. *Cancer Res.* **61**:1080–1088 (2001).
52. B. W. Tillman, T. D. de Gruijl, S. A. Luykx-de Bakker, R. J. Scheper, H. M. Pinedo, T. J. Curiel, W. R. Gerritsen, and D. T. Curiel. Maturation of dendritic cells accompanies high-efficiency gene transfer by a CD40-targeted adenoviral vector. *J. Immunol.* **162**:6378–6383 (1999).
53. B. W. Tillman, T. L. Hayes, T. D. DeGruijl, J. T. Douglas, and D. T. Curiel. Adenoviral vectors targeted to CD40 enhance the efficacy of dendritic cell-based vaccination against human papillomavirus 16-induced tumor cells in a murine model. *Cancer Res.* **60**:5456–5463 (2000).
54. S. K. Nair, D. Boczkowski, M. Morse, R. I. Cumming, H. K. Lyerly, and E. Gilboa. Induction of primary carcinoembryonic antigen (CEA)-specific cytotoxic T lymphocytes in vitro using human dendritic cells transfected with RNA. *Nat. Biotechnol.* **16**:364–369 (1998).
55. M. A. Chattergoon, J. J. Kim, J. S. Yang, T. M. Robinson, D. J. Lee, T. Dentchev, D. M. Wilson, V. Ayyavoo, and D. B. Weiner. Targeted antigen delivery to antigen-presenting cells including dendritic cells by engineered fas-mediated apoptosis. *Nat. Biotechnol.* **18**:974–979 (2000).
56. B. Pulendran, J. L. Smith, M. Jenkins, M. Schoenborn, E. Maraskovsky, and C. R. Maliszewski. Prevention of peripheral tolerance by a dendritic cell growth factor: flt3 ligand as an adjuvant. *J. Exp. Med.* **188**:2075–2082 (1998).
57. B. Pulendran, J. Banchereau, E. Maraskovsky, and C. Maliszewski. Modulating the immune response with dendritic cells and their growth factors. *Trends Immunol.* **22**:41–47 (2001).
58. S. Raychaudhuri and K. L. Rock. Fully mobilizing host defense: building better vaccines. *Nat. Biotechnol.* **16**:1025–1031 (1998).
59. C. Sedlik, R. Perraut, B. Bonnemains, and C. Leclerc. Antigens linked to synthetic microspheres induce immune responses in primates in the absence of adjuvant. *Immunobiology* **195**:105–118 (1996).
60. S. Babiuk, M. Baca-Estrada, L. A. Babiuk, C. Ewen, and M. Foldvari. Cutaneous vaccination: the skin as an immunologically active tissue and the challenge of antigen delivery. *J. Control. Release* **66**:199–214 (2000).
61. D. Kaiserlian and N. Etchart. Entry sites for oral vaccines and drugs: A role for M cells, enterocytes and dendritic cells? *Semin. Immunol.* **11**:217–224 (1999).