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Review

# Designing Vaccines Based on Biology of Human Dendritic Cell Subsets

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The effective vaccines developed against a variety of infectious agents, including polio, measles, and hepatitis B, represent major achievements in medicine. These vaccines, usually composed of microbial antigens, are often associated with an adjuvant that activates dendritic cells (DCs). Many infectious diseases are still in need of an effective vaccine including HIV, malaria, hepatitis C, and tuberculosis. In some cases, the induction of cellular rather than humoral responses may be more important because the goal is to control and eliminate the existing infection rather than to prevent it. Our increased understanding of the mechanisms of antigen presentation, particularly with the description of DC subsets with distinct functions, as well as their plasticity in responding to extrinsic signals, represent opportunities to develop novel vaccines. In addition, we foresee that this increased knowledge will permit us to design vaccines that will reprogram the immune system to intervene therapeutically in cancer, allergy, and autoimmunity.

## Introduction

Vaccines can be preventive or therapeutic. The word vaccination was first used by Edward Jenner in 1796 to describe the injection of smallpox derived from cows (*L. vaccae*, cow). Louis Pasteur discovered that animals and people could be protected against disease after exposure to attenuated microbes. Most, if not all, preventive vaccines are designed to initiate protective humoral immune responses. However, many pathogens, for which no efficient vaccines are available, are still affecting mankind with diseases such as human immunodeficiency virus (HIV)-induced acquired immune deficiency syndrome, plasmodium-induced malaria, virus-induced hepatitis C, and *Mycobacterium*-induced tuberculosis. Most of these appear to be chronic diseases for which it is thought that strong cellular immunity, in particular cytotoxic T cells, is necessary to eliminate the cells that are infected with the causative agent. Thus, therapeutic vaccines are needed to eliminate existing disease as much as prophylactic vaccines that might block the initial infection. Vaccines have yet to be developed in noninfectious settings, where they have the potential to prevent and treat cancer, allergy, and chronic inflammation.

A more detailed understanding of the mechanisms leading to strong cellular immunity is necessary to enable rational approaches to vaccine design. Two recent conceptual breakthroughs in this regard have been (1) our understanding that dendritic cells (DCs) play a pivotal role in initiating the immune response to foreign antigens (Figure 1) and (2) the realization that adjuvants act primarily because they are DC activators. Preventive vaccines are based on the concept of transitioning from no immunity to immunity by generating new $CD4^+$ or $CD8^+$ T effector cells by "priming" a new immune response. Therapeutic vaccines in chronic infections (or cancer) have two objectives: one is priming whereas the other is the modulation or reprogramming of memory cells, i.e., to transition from one type of immunity to another (e.g., regulatory to cytotoxic). These two types of vaccination might necessitate distinct approaches, facilitated by exploiting the diversity of DCs including their different subsets and functional plasticity.

## The Challenge of Eliciting the Right Immune Response

The efficacy of vaccination is directly linked to the type and the quality of immune responses elicited by a particular vaccine. Indeed, generating the right class of immune response can be a matter of life and death, perhaps best illustrated by leprosy where the indolent tuberculoid form of the disease is characterized by a protective type 1 T cell (Th1 cell) response, whereas the lepromatous form induces an often lethal type 2 (Th2 cell) response.

The quality of $CD4^+$ T cell immunity is essential for the quality of effector cells such as antibody-secreting plasma cells and cytotoxic $CD8^+$ T cells. $CD4^+$ T cells also appear necessary for the efficient generation of memory $CD8^+$ T cells (Janssen et al., 2003; Shedlock and Shen, 2003; Sun and Bevan, 2003). $CD4^+$ T cells display a broad spectrum of phenotypes, which is probably due to the priming by antigen-presenting cells (APCs), most often DCs (Figure 2; reviewed in Bluestone et al., 2009). Thus, in response to intracellular microbes, such as viruses and certain bacteria, $CD4^+$ T helper cells differentiate into Th1 cells, which secrete interferon-$\gamma$ (IFN-$\gamma$). In contrast, extracellular pathogens induce the development of Th2 cells, whose cytokines (interleukin-4 [IL-4], IL-5, IL-10, and IL-13) direct immunoglobulin E- and eosinophil-mediated destruction of the pathogens (Mosmann et al., 1986).

DCs regulate $CD4^+$ T cell differentiation through a variety of molecules that belong to three major families: IL-12, TNF, and B7. The IL-12 family includes IL-12p70, which controls Th1 cell

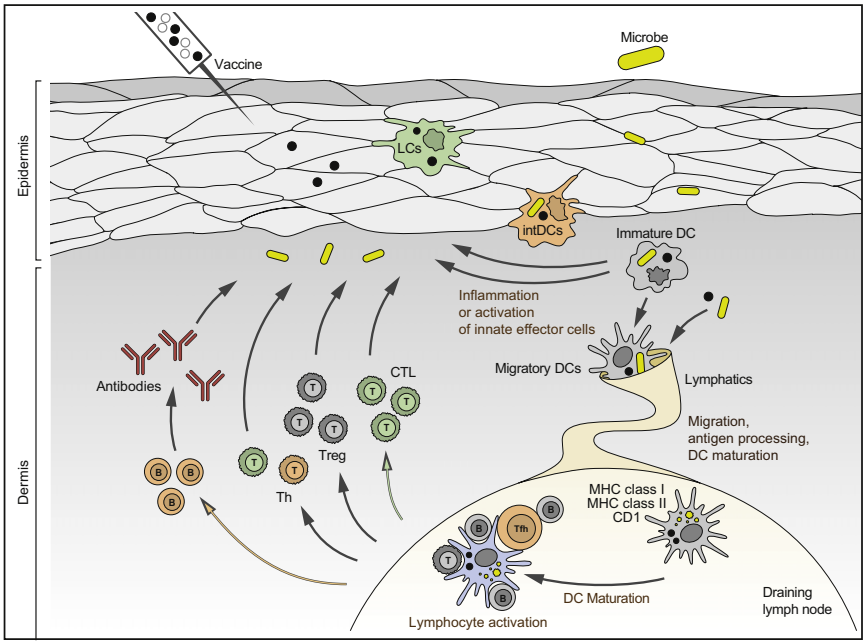


**Figure 1. Dendritic Cells**

DCs reside in the tissue where they are poised to capture antigens, be it microbes or vaccines. DCs recognize microbes (vaccines) and secrete cytokines (e.g., IFN-α) directly through pattern recognition receptors or indirectly through stromal cells that sense microbes (vaccines). Cytokines secreted by DCs in turn activate effector cells of innate immunity such as eosinophils, macrophages, and NK cells. Activation triggers DC migration toward secondary lymphoid organs and simultaneous activation (maturation). These migratory DCs display antigens in the context of classical MHC class I and class II or nonclassical CD1 molecules, which allow selection of rare antigen-specific T lymphocytes. Activated T cells drive DCs toward their terminal maturation, which induces further expansion and differentiation of lymphocytes. Activated T lymphocytes traverse inflamed epithelia and reach the injured tissue, where they eliminate microbes and/or microbe-infected cells. B cells, activated by DCs and T cells, differentiate into plasma cells that produce antibodies against the initial pathogen. Antigen can also drain into lymph nodes without involvement of peripheral tissue DCs and be captured and presented by lymph node-resident DCs. Antigen capture by interstitial DCs (intDCs; orange) will preferentially lead to generation of humoral immunity, whereas antigen capture by Langerhans cells (LCs; green) will preferentially lead to generation of cellular immunity.

responses (Macatonia et al., 1995); IL-23, which controls inflammatory $CD4^+$ T cells secreting IL-17 (Th17 cells) (Weaver et al., 2007); and IL-27, which appears to control IL-10 (Kastelein et al., 2007). Depending on the nature and time course of activation (maturation) by different agonists, DCs can express different molecules from the B7 family: CD80 (B7-1), CD86 (B7-2), ICOS-ligand, PD-L1 (B7-H1), PD-L2 (B7-DC), B7-H3, and B7-H4 (Chen, 2004; Greenwald et al., 2005). The B7 family includes members that can stimulate immune responses and others that can inhibit them (Chen, 2004). For instance, CD80 and CD86 bind to both CD28 and CTLA-4. Whereas CD28 delivers signals for T cells to become effector cells, CTLA-4 delivers inhibitory signals that suppress their functions (Krummel and Allison, 1995). Furthermore, through its mode of action, one molecule might promote both the effector and the regulatory response, as exemplified by the ICOS ligand. Indeed, ICOS: ICOS ligand interaction helps the generation of regulatory T (Treg) cells (Ito et al., 2007) but also appears important in the stimulation of effector T cells and T cell-dependent B cell responses (Hutloff et al., 1999). A member of the TNF family, OX40L (which binds to OX40), shuts down the generation of IL-10-producing $CD4^+$ type 1 regulatory T (Tr1) cells by DCs (Ito et al., 2006) but induces the differentiation of proinflammatory Th2 cells secreting TNF and IL-13. As we will discuss later, DCs and IL-12 are also essential regulators of another type of helper T cells, so-called T follicular helper (Tfh) cells, which in turn regulate humoral immunity (Schmitt et al., 2009).

A key cell population involved in the regulation of immune responses and homeostasis are Treg cells, which include two major subsets: thymus-derived naturally occurring Treg cells and periphery-induced Treg cells (Sakaguchi et al., 2010). Peripherally induced Treg cells are thought to be derived from naive $CD4^+$ T cells and include Tr1 cells, which mainly produce IL-10 (Roncarolo et al., 2001a), and Th3 cells, which mainly produce TGF-β (Fukaura et al., 1996). In turn, TGF-β1 synergizes with IL-21 to generate IgA-plasmablasts, thereby playing a critical role in the development of mucosal immunity (Dullaers et al., 2009). The functional specialization of DC subsets in governing the differentiation of distinct types of Treg cells is currently a subject of active investigation. Peripheral Treg cells are generated by DCs that exist at the steady state, i.e., DCs that have not been activated by microbial stimuli or inflammatory mediators (Roncarolo et al., 2001b; Yamazaki et al., 2006). These DCs may not simply be unstimulated or immature. Activation of the Wnt and β-catenin signaling pathway in DCs has been shown to promote induced Treg cell production, at least in the mouse (Jiang et al., 2007). Similarly, in the thymus, production of thymic stroma lymphopoietin (TSLP) is essential for selection of naturally occurring $CD4^+CD25^{hi}$ Treg cells (Watanabe et al., 2005).

## Licensing Dendritic Cell Function: A Word on DC "Maturation"

DCs exist in distinct functional states including resting and activated, also known as immature and mature. This is a key feature of DC biology and relates to the process of DC "maturation," classically described as the morphological and functional alterations associated with the activation of DCs by microbial stimuli (e.g., via Toll-like receptor [TLR] agonists) (Trombetta and Mellman, 2005). Under steady-state conditions, DCs in peripheral tissues are most often described as being "immature," a phenotype characterized by the localization of MHC class II molecules to the late endosome-lysosomal compartment, a low surface expression of costimulatory molecules, low expression of chemokine receptors that trigger migration (e.g., CCR7), and an inability to release T cell-directed immunostimulatory cytokines (Trombetta and Mellman, 2005). Particularly adept at

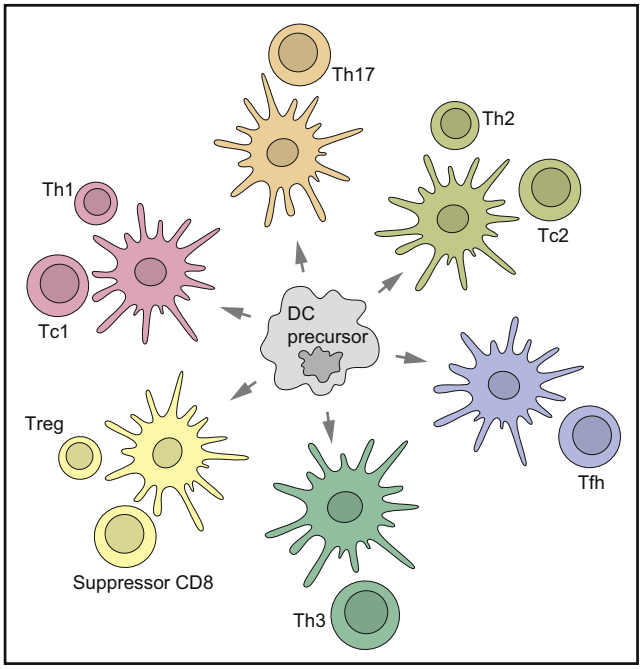


**Figure 2. Distinct DC Subsets Generate Distinct Types of T Cell Immunity**

DC system has two cardinal features: (1) subsets and (2) plasticity. This yields distinct types of immunity, thereby allowing DCs to cope with protection against a variety of microbes and maintenance of tolerance to self. Understanding these two features is fundamental to develop vaccines that elicit the desired type of immune responses.

endocytosis, immature DCs are often associated with antigen uptake and sequestration, but not with antigen processing, the stable accumulation of peptide-MHC complexes, or their efficient presentation to T cells (Trombetta and Mellman, 2005). Maturation, as triggered by TLR agonists, upregulates surface MHC class II and costimulatory molecules on the DCs (Trombetta and Mellman, 2005) as well as promoting their migration to draining lymph nodes. It also enhances the ability of the DCs to interact with antigen-specific T cells, more efficient antigen processing and presentation, and cytokine release (Lanzavecchia and Sallusto, 2001). Thus, it is DC maturation, triggered by adjuvants, that links the innate and antigen-specific arms of the immune response and thus allows the adaptive immunity to launch the response against a specific antigen (Steinman et al., 2003). Because agonist receptors, such as TLRs, are differentially expressed by different DC subsets and because different receptors may trigger qualitatively distinct forms of maturation (e.g., different patterns of cytokine release), understanding and accounting for DC maturation will be a key component of any attempt at rational vaccine design because it will determine the adjuvant used.

Maturation is a simple concept rendered complex by the likelihood that not all mature (or activated) DCs are equivalently immunogenic (Figure 3). For example, under steady-state conditions, particularly in lymphoid tissue, one can find DC populations that display at least some of the features of mature DCs (e.g., elevated surface costimulatory molecules) despite the absence of overt inflammation or infection. The functional significance of these cells is unknown but it is not unreasonable to suspect that tolerogenic DCs may have to acquire the antigen presentation, migratory, and T cell interaction capacity of mature DCs in order to induce antigen-specific Treg cells or induce anergy or T cell apoptosis at high efficiency. As mentioned above, the priming of Treg cells either in the thymus or in the periphery may require activation by endogenous mediators such as TSLP or Wnt, respectively (Watanabe et al., 2005; Manicassamy et al., 2010). Whether these mediators induce morphologically recognizable maturation in vivo is likely but not known. However, it is clear that resting or immature DCs can or must be "activated" in some way to induce T cell tolerance; hence, it is inaccurate to assume that the relevant steady-state DCs are "immature" or resting.

Virtually all DC subsets identified thus far, and discussed below, are capable of some form of activation, even if not all of them exhibit the dramatic cellular reorganizations observed for myeloid and monocyte-derived DCs. Plasmacytoid DCs, for example, do not dramatically relocalize their MHC class II molecules from lysosomes to the plasma membrane, but respond functionally (e.g., by interferon secretion) to a range of TLR agonists to facilitate immunity (Siegal et al., 1999). Because "mature" is usually associated with DCs that have undergone a morphological transition, we will use the more general term "activated" to describe the responses of DC subsets to adjuvants or endogenous activators when their phenotypic status (particularly in vivo) is unclear.

## Human Dendritic Cell Subsets

Although activation or maturation is a key factor determining DC function, the increasing number of distinct DC subsets being recognized indicates that the distribution of labor among DC subtypes is likely to be an equally important aspect of how DCs regulate T cell priming. We will concentrate on DC subsets that are associated with immunity; however, even in peripheral tolerance induction, some subsets may be more effective than others (Siddiqui et al., 2010). Other subsets, notably DCs in B cell follicular regions, may be most adept at interacting with B cells, inducing humoral immunity to unprocessed soluble antigen trapped by these DCs (Wykes et al., 1998).

### *DC Subsets in Human Blood*

The evolution of knowledge of DC subsets has followed parallel tracks in mice and humans, and understanding them has become a major focus for many investigators over the past 15 years. Humans and mice display two major DC types: myeloid DCs (mDCs, also called conventional or classical DCs [cDCs]) and plasmacytoid DCs (pDCs). In mice, splenic mDCs were originally shown to comprise two major mDC subsets with marked differences in biological function: $CD8\alpha^{+}CD11b^{-}$ "lymphoid" DCs and $CD8\alpha^{-}CD11b^{+}$ "myeloid" DCs. $CD8\alpha^{+}$ DCs are able to produce large amounts of IL-12 and polarize naive $CD4^{+}$ T cells toward the Th1 cell phenotype, whereas $CD8\alpha^{-}$ DCs preferentially induce Th2 cell responses (Maldonado-López et al., 1999). Although the study of mouse DC subsets can make important contributions, it is crucial to do such studies with human cells because subtle but highly relevant differences exist between the human and mouse immune systems (Mestas and Hughes, 2004). Thus, to successfully generate human vaccines, we need to understand the diversity and biology of human DC

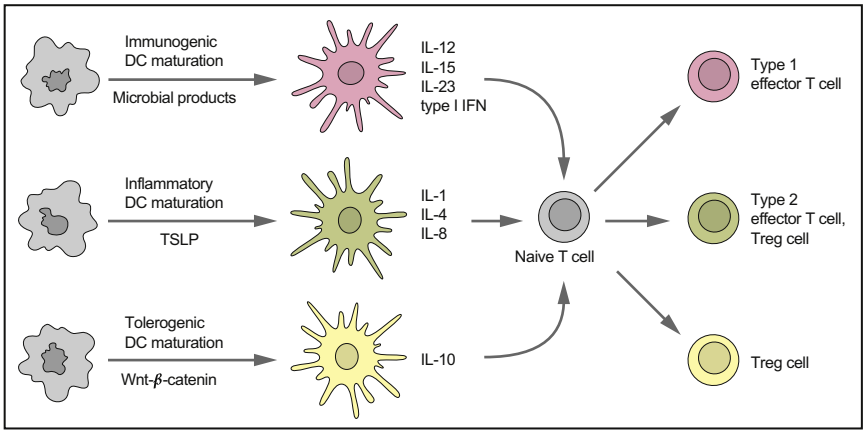


**Figure 3. Many Roads Lead to DC Maturation**

DCs exist in distinct functional states: resting and activated, or immature and mature. Depending on the signal, DCs will undergo activation/maturation, the quality of which will determine the type of elicited adaptive immunity.

subsets. DC subsets in the human blood can be distinguished by differential expression of three surface molecules: BDCA-1 (CD1c), BDCA-2 (CD303), and BDCA-3 (CD141) (Dzionek et al., 2000).

BDCA-2$^+$ pDCs are considered the front line in antiviral immunity owing to their capacity to rapidly produce high amounts of type I interferon in response to viruses (Siegal et al., 1999). They also express high amounts of IL-3R$\alpha$ chain (CD123) and ILT-7 (Cao et al., 2006). pDCs are composed of at least two subsets with different functional properties (Matsui et al., 2009). They recognize viral components and self nucleic acids through TLR7 and TLR9, and possibly other as-yet-unidentified receptors. In their resting state, pDCs might play an important role in tolerance, including oral tolerance (Liu, 2005). The pDC presents three remarkable cell biological features to counteract viral infection: an extensive ER compartment that facilitates high-capacity secretion of antiviral factors, including type I interferons; an early endosomal compartment containing MHC class I molecules that appears to permit direct vesicular MHC class I loading for immediate activation of memory cytotoxic CD8$^+$ T cells (Di Pucchio et al., 2008); and a late endosomal compartment containing MHC class II molecules, similar to that found in mDCs, which facilitates viral antigen presentation to CD4$^+$ T cells. Thus, in both the MHC class I and class II pathways, pDCs may permit a rapid initial response to viral infections by utilizing presynthesized stores of MHC class I and II. In addition to their specialized role in the innate immune response to viruses (e.g., type I IFN release), pDCs are uniquely capable of rapidly expanding viral antigen-specific CD8$^+$ T effector cells (Di Pucchio et al., 2008). Thus, pDCs are poised to control the progress of a virus infection through nonspecific blockade of viral replication by type I IFN and the specific stimulation of adaptive antiviral responses via cytotoxic CD8$^+$ T cells. pDCs are also critical for the generation of plasma cells and antibody responses (Jego et al., 2003). There, two mechanisms are employed to amplify B cell responses: (1) type I IFN and IL-6 upon viral stimulation (Jego et al., 2003) and (2) type I IFN-independent mechanism that is based on their stable expression of CD70 upon CpG activation (Shaw et al., 2010). Finally, by virtue of their special capacity for secreting type I IFN, stimulating pDCs may provide an endogenous adjuvant that could promote the immunogenic maturation of other DC populations. Thus, strategies designed to prime pDCs may form the basis of a next generation of antiviral vaccines.

In human blood there are two types of mDCs distinguished by reciprocal expression of BDCA-1 (CD1c) and BCDA-3 (CD141). Human CD141$^+$ DCs represent the human counterpart of mouse CD8$^+$ DCs. Indeed, they share with mouse CD8$^+$ DCs the high capacity to capture exogenous antigens for presentation on HLA class I molecules ("cross-presentation"), typically reserved for the presentation of peptides from endogenous antigens. CD141$^+$ DCs also share the expression of chemokine receptor XCR1 and of adhesion molecule Necl2. Both human CD141$^+$ DCs and mouse CD8$^+$ DCs utilize XCR1 to migrate in response to the specific ligand XCL1, which is produced by NK cells and activated CD8$^+$ T cells (Bachem et al., 2010; Crozat et al., 2010). Necl2 binds to class I-restricted T cell-associated molecule (CRTAM), a cell surface protein primarily expressed by NK cells, NK-T cells, and activated CD8$^+$ T cells. Thus, mouse CD8$^+$ DCs and human CD141$^+$ DCs appear well equipped for generation of CD8$^+$ T cell immunity. In the mouse, gene ablation studies have also shown that the CD8$^+$ subset plays a disproportionately important role in cross-presentation (Shortman and Heath, 2010).

The identification of the human counterpart of mouse CD8$^+$ DCs opens the possibility to translate into humans the knowledge generated in the mouse. One should, however, translate mouse data into clinical applications with a critical mind, because 65 million years of independent evolution have brought in many nuances that distinguish the human and the mouse immune systems (Mestas and Hughes, 2004). For example, other human DCs such as epidermal LCs (Klechevsky et al., 2008) can also cross-present antigens. Thus, it remains to be determined whether and how CD141$^+$ blood mDCs are related to cutaneous mDCs subsets and how all those mDC subsets cooperate in shaping the adaptive immunity. Blood CD1c$^+$ DCs also display a capacity to cross-present antigens and to secrete IL-12 (Jongbloed et al., 2010; Poulin et al., 2010). Also, even if CD141$^+$ DCs are far more adept at cross-presentation than other DC subsets, "mass action" is a consideration because CD141$^+$ DCs represent only a small fraction (~2%) of all DCs, at least in the blood. Thus, how these distinct blood mDC subsets contribute to shaping immunity remains to be established.

### *DC Subsets in Human Skin*

In human skin, at least two different mDC subsets have been characterized: epidermal Langerhans cells (LCs) and dermal interstitial DCs (dermal DCs) (Valladeau and Saeland, 2005). Over the years, dermal DCs were further subdivided into at least two subsets: CD1a$^+$ DCs and CD14$^+$ DCs (Valladeau and

Saeland, 2005). The presence of two dermal DC subsets was also reported in mice that display a Langerin (CD207) subset in the dermis (Merad et al., 2008). Epidermal LCs and dermal $CD14^+$ DCs express different sets of molecules. In particular, $CD14^+$ DCs express a large number of surface C-type lectins including DC-SIGN, DEC-205, LOX-1, CLEC-6, Dectin-1, and DCIR. In contrast, LCs express the lectins Langerin and DCIR. Furthermore, whereas dermal $CD14^+$ DCs express, at RNA level, a wide range of TLRs, including TLR-2, 4, 5, 6, 8, and 10 (Klechevsky et al., 2009; van der Aar et al., 2007), LCs exhibit a more restricted TLR expression including TLR-1, 3, 6, and 10.

Studies suggest that human $CD14^+$ DCs induce naive T cells to differentiate into cells with properties of Tfh cells (Klechevsky et al., 2008). Thus, $CD4^+$ T cells primed by $CD14^+$ DCs are able to induce naive B cells to produce larger amounts of IgM than those primed with LCs. Remarkably, only $CD4^+$ T cells primed by $CD14^+$ DCs induce naive B cells to switch isotypes toward IgG and IgA. Furthermore, $CD4^+$ T cells primed by $CD14^+$ DCs secrete the chemokine CXCL13, a typical chemokine secreted by Tfh cells. Taken together, these data suggest that human dermal $CD14^+$ DCs are specialized for the development of humoral responses (Klechevsky et al., 2008; Ueno et al., 2007). Along these lines, human monocyte-derived DCs activated with ligands of TLR-4, 5, and 7-8, heat-inactivated bacteria, or CD40 ligand efficiently induce naive $CD4^+$ T cells to become IL-21 producers, which in turn induce B cells to produce Ig, in the process mediated predominantly by IL-12 (Schmitt et al., 2009).

LCs induce more robust proliferation of naive allogeneic $CD4^+$ and $CD8^+$ T cells when compared to $CD14^+$ DCs (Klechevsky et al., 2008). LCs are also more efficient in cross-presenting peptides from protein antigens to $CD8^+$ T cells and prime $CD8^+$ T cells of high avidity when compared to $CD14^+$ DCs. $CD8^+$ T cells primed by LCs acquire more potent cytotoxicity than those primed by $CD14^+$ DCs and are able to efficiently kill target cells, including tumor cell lines that express peptide-HLA complex only at low amounts (Klechevsky et al., 2008). Dermal $CD14^+$ DCs showed a poor ability to induce differentiation of CTL effectors. This is not due to the inability to generate peptide-MHC class I complexes, but rather the inability to induce the expression of the cytotoxic effector molecules (granzymes A and B and perforin) on the differentiating T cells. The limited ability of $CD14^+$ DCs to cross-present proteins such as influenza matrix protein is not due to a lower ability to process proteins in general; these cells are indeed more potent at processing MHC class II-restricted peptides from tetanus toxoid. The mechanistic basis for why some DCs are more efficient at cross-presentation remains an important unknown. One possibility is that the increased concentrations of proteolytic enzymes found within the endocytic compartments of monocyte-derived DCs destroy internalized antigens before they have the chance to egress into the cytosol (McCurley and Mellman, 2010). In general, an attenuated capacity for proteolysis is a key feature in enhancing antigen processing and presentation by DCs, in both the MHC class I and class II pathways (Delamarre et al., 2005; Trombetta and Mellman, 2005).

Although $CD14^+$ DCs educate naive $CD4^+$ T cells to become Tfh-like cells, LCs polarize naive $CD4^+$ T cells into cells secreting Th2 cell-type cytokines such as IL-4, IL-5, and IL-13. This is consistent with mouse studies showing the preferential induction of Th2 cell responses upon delivery of an antigen to the LC-rich epidermis (Alvarez et al., 2005).

For many years, LCs have been viewed as a paradigm population in DC biology. Induction of potent CTL response by LCs is observed in mouse studies by subcutaneous injections of peptide-loaded epidermal LCs (Celluzzi and Falo, 1997). Mouse LCs can actually cross-present antigens to $CD8^+$ T cells in vivo (Stoitzner et al., 2006). In contrast, several mouse studies, for example models using herpes simplex virus (HSV), have questioned the contribution of LCs to the induction of antigen-specific responses in vivo. These studies attribute the HSV-specific immunity to $CD8\alpha^+$ DCs, rather than to LCs (Allan et al., 2003). Further ex vivo studies showed that dermal $CD103^+$ DCs but not dermal $CD11b^+$ nor LCs were able to present antigens to naive TCR-transgenic $CD8^+$ T cells ex vivo (Bedoui et al., 2009). In contrast, all DCs were able to present viral antigens to $CD4^+$ T cells (Bedoui et al., 2009). These results suggest that although the three cutaneous DC populations acquired viral antigens, only $CD103^+$ DCs were able to present viral antigens to $CD8^+$ T cells. However, it remains to be determined whether these differences with regard to the function of LCs between mice and humans derive from the differences in their immune systems. One further unknown is the susceptibility of these DC subsets to virus infection, which may substantially modulate antigen-presenting function.

### *Humoral versus Cellular Immunity Regulated by Two mDC Subsets*

Collectively, we hypothesize that two different components of adaptive immunity, i.e., humoral and cellular, are preferentially regulated by different mDC subsets, at least in the skin. Thus, although humoral immunity is preferentially regulated by $CD14^+$ dermal DCs, cellular immunity is preferentially regulated by LCs (Figure 4). This idea is also supported by mouse studies showing that dermal DCs upon activation migrate into the outer paracortex just beneath the B cell follicles, whereas LCs migrate into the T cell-rich inner paracortex (Kissenpfennig et al., 2005). Another human skin DC subset, dermal $CD1a^+$ DCs, are functionally intermediate between LCs and $CD14^+$ DCs in our hands. Whether this DC subset shows a unique asset in the regulation of immune responses remains to be addressed. It will also be important to understand whether this paradigm applies to DCs localized to other peripheral and lymphoid tissues in humans.

## Plasticity of DCs and Their Precursors as Key Determinants of Immunity

In addition to subsets with functional specialization, DCs and their precursors (monocytes) are endowed with functional plasticity (Figures 2 and 3). DC plasticity needs to be considered at three levels: (1) response to microbial signals, (2) sensing of tissue-derived factors, and (3) reciprocal interaction with other immune cells.

Upon microbial invasion, DCs undergo an initial activation and maturation process that includes (1) direct signaling by microbial products and (2) microenvironmental signals delivered by surrounding cells responding to the microbes (Reis e Sousa, 2006; Trombetta and Mellman, 2005). Pathogen-derived signals transform resting or immature DCs into activated or mature cells able to launch adaptive immunity. Microbial products can deliver

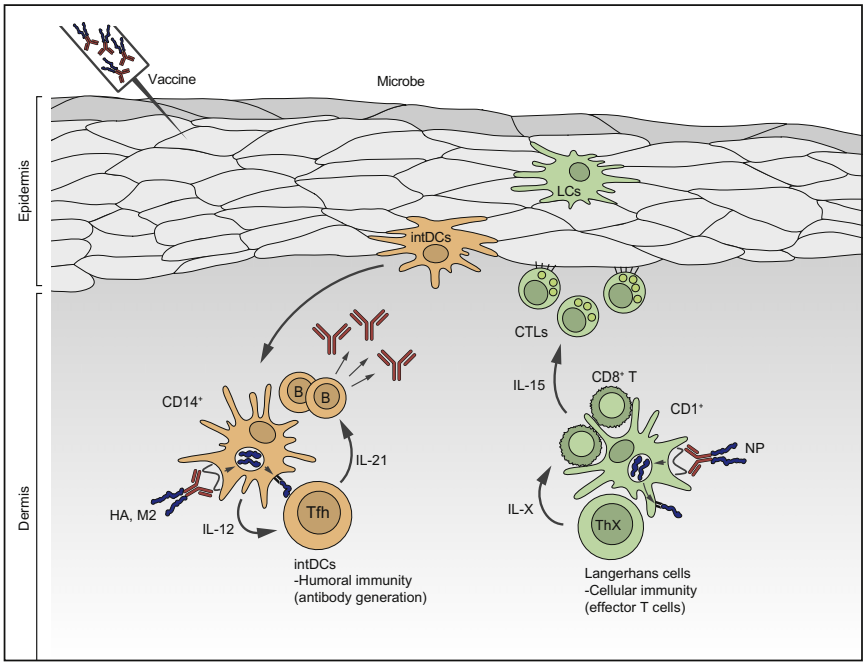


**Figure 4. Understanding Human Myeloid Dendritic Cell Subsets for the Rational Design of DC-Targeting Vaccines**

Novel vaccines rely on rational immunological approaches and aim at activating both the cellular and the humoral arm. We envision that targeting antigens and activation of distinct mDC subsets, with different specializations, will result in the generation of a broad and long-lived immune protection. Thus, the most efficient vaccines might be those that will target both LCs and dermal $CD14^+$ DCs, thereby allowing the maximal stimulation of cellular and humoral immune responses and the generation of long-term memory protection. Here we illustrate this concept by using vaccines against influenza antigens hemagglutinin (HA) and viral envelope M2 protein to which it is desirable to elicit antibody responses and nucleoprotein (NP), which is expected to provide CD8 epitopes on infected cells, thereby requiring vaccines that elicit strong cytotoxic $CD8^+$ T cell responses. In this scenario, vaccines targeting HA or M2 antigens to interstitial DCs would elicit humoral response, which are amplified by IL-21-secreting Tfh cells. Vaccines targeting NP to Langerhans cells would favor $CD8^+$ T cell immunity. The phenotype of $CD4^+$ T cells (ThX) that provide help for cytotoxic $CD8^+$ T cells and a cytokine that is involved in this process (IL-X) remain to be defined.

signals via several molecules, PPRs, belonging to four major families: (1) C-type lectins, (2) TLRs, (3) NOD-like receptors, and (4) RIG-I-like receptors. These signals can differentially modulate DC function, consequently yielding distinct immune responses (Manicassamy and Pulendran, 2009; Takeuchi and Akira, 2010). For example, some C-type lectins have signaling motifs in their cytoplasmic regions and deliver activation or suppression signals (Reis e Sousa, 2006). Similar to TLR expression, CLR expression differs between human and mouse (Flornes et al., 2004). CLRs are also receptors for endogenous ligands. For example, Mincle and Clec9a (DNGR-1) recognize damaged cells, Mincle by detecting small nuclear ribonucleoprotein (Brown, 2008), which is released from damaged cells, and Clec9a by detecting as yet unidentified preformed ligand(s) exposed on necrotic cells (Sancho et al., 2009).

Similarly, different TLRs deliver different activation signals to DCs (Manicassamy and Pulendran, 2009). Thus, *Escherichia coli* lipopolysaccharide (LPS) stimulates DCs through TLR4, inducing a Th1 cell response by IL-12 secretion, whereas *Porphyromonas gingivalis* LPS activates DCs through TLR2, inducing DCs to secrete IL-10, and eventually resulting in Th2 cell development (Manicassamy and Pulendran, 2009).

Cytoplasmic sensors include RIG-I-like receptors (the intracellular receptors for RNA viruses) and NOD-like receptors (NLRs), which are thought to recognize microbial components (Takeuchi and Akira, 2010). NLRs, such as NALP1, NALP3, IPAF, and NAIP5, are components of a molecular complex called the inflammasome (Schroder and Tschopp, 2010). The inflammasome cleaves substrates, such as pro-IL-1$\beta$ and pro-IL-18, to produce mature proteins. NOD1/2 are expressed in the cytosol of macrophages and DCs, and NALP1 is absent in germinal center and interstitial DCs while it is highly expressed in LCs within mucosal surfaces and skin (Schroder and Tschopp, 2010).

The concept of plasticity or flexibility of the DC system is further exemplified by monocytes and their response to environmental signals. Thus, different cytokines skew the in vitro differentiation of monocytes into DCs with different phenotypes and function. This might in fact reflect the inflammatory pathway of DC recruitment and generation in vivo (Domínguez and Ardavín, 2010; Geissmann et al., 2010). For example, when activated (for example by GM-CSF) monocytes encounter IL-4, they will yield IL-4-DCs (Romani et al., 1994). By contrast, after encounter with IFN-$\alpha$, TNF, or IL-15, activated monocytes will differentiate into IFN-DCs (Paquette et al., 1998), TNF-DCs, or IL-15-DCs (Mohamadzadeh et al., 2001), respectively. This spectrum of DCs represents immunostimulatory DCs although their in vivo counterparts and precise identities are unknown. Furthermore, it has been argued that cytokine-driven DCs might not be as potent in the generation of adaptive immunity as are the DCs triggered directly via microbial signals through PRRs (Joffre et al., 2009).

Similarly, there is a whole repertoire of DCs that have been produced in vitro that exhibit immunoregulatory or tolerogenic functions, for example DCs generated by culturing monocytes with IL-10 (Levings et al., 2005) or DCs generated in the presence of vitamin A (Zapata-Gonzalez et al., 2007) or vitamin D3 (Penna and Adorini, 2000), or DCs activated by E-cadherin-mediated signaling (Jiang et al., 2007). Should such diversity exist in vivo, these DC populations might well be important in the context of DCs' role in maintaining peripheral tolerance. Tissue-localized mDCs are also polarized by other cells and their products, including IFN-$\alpha$ from pDCs, IFN-$\gamma$ from $\gamma\delta$ T cells and NK cells, IL-4 and TNF from mast cells, IL-15 and TSLP from stromal cells, IL-10 from lymphocytes, and Wnt ligands from various cellular sources (reviewed in Cheng et al., 2010; Ueno et al., 2010). In principle, these distinct DCs will induce distinct types of T cell immunity or tolerance.

Such plasticity is associated with distinct signaling pathways as shown by a recent study (Arima et al., 2010). There, TSLP via activation of NF-κB leads DCs to express OX40L, allowing the induction of Th2 cell differentiation, whereas the activation of signal transducer and activator of transcription 6 (STAT6) triggered DCs to secrete chemokines necessary for the recruitment of Th2 cells. In addition, TSLP signaling limited the activation of STAT4 and interferon regulatory factor 8 (IRF-8), which are essential factors for the production of the Th1 cell-polarizing cytokine IL-12. This Th1 cell-inducing pathway was instead activated by TLRs and CD40 ligand. Thus, the functional plasticity of DCs relies on elaborate signal codes that are generated by different stimuli. As alluded to above, the DC activation or maturation process is more sophisticated than just licensing DCs for T cell stimulation; it enables DCs to sense their environment and to assume an activated phenotype that carefully instructs the qualitative nature of the T cell responses induced.

DCs also have a reciprocal interaction with innate immune cells. The interaction of DCs with NK, NKT, and γδ T cells can occur in the periphery and the secondary lymphoid organs (reviewed in Münz et al., 2005). A recent mouse study suggested that the activation of NK cells is totally dependent on the interaction with DCs at the secondary lymphoid organs (Lucas et al., 2007). Activated NK cells enhance their cytotoxicity and capacity to secrete IFN-γ, which render DCs to induce type 1 responses (Münz et al., 2005). Mature DCs also activate NKT and γδ T cells, inducing the secretion of IFN-γ and IL-4 from NKT cells (Hermans et al., 2003) and IFN-γ and TNF-α from γδ T cells (Leslie et al., 2002). In particular, activated NKT cells acquire the capacity to kill tumor cells (Smyth et al., 2002). In return, CD40L expressed on NKT cells induces the strong activation of DCs (Münz et al., 2005).

Thus, subsets and plasticity allow DCs to cope with the challenges of their environment. These two features also dictate the quality of the response to vaccine adjuvants and can be harnessed for improved vaccination.

## Targeting of Dendritic Cell Subsets to Improve Vaccines

Translating the accumulating knowledge on DC subsets and their unique functional attributes into the design of novel vaccines is becoming an exciting topic in human immunology. Active immunization has long been a successful strategy for the prevention of infectious diseases. The question now is how to capitalize on our new understanding of DCs to improve vaccines to the point where they can now also be used more effectively as therapeutic strategies.

Antigens can be delivered directly to DCs in vivo by using various types of fusion proteins including cytokines (for example GM-CSF), chemokines, and toxins, or more specifically antibodies against specific DC surface receptor(s). Studies in mice demonstrate that the specific targeting of antigen to DCs in vivo results in considerable potentiation of antigen-specific $CD4^+$ and $CD8^+$ T cell immunity. The induction of immunity is observed only when a DC maturation signal was provided (Bonifaz et al., 2002; Hawiger et al., 2001); otherwise, tolerance ensued (Hawiger et al., 2001). Furthermore, in vivo targeting of murine DC subsets revealed intrinsic differences in antigen processing and presentation of different populations (Dudziak et al., 2007). As discussed earlier, the $CD8^+CD205^+$ population was found to be more adept at cross-presentation of exogenous antigen on MHC class I than the $CD8^-33D1^+$ DC population, which was somewhat more efficient at MHC class II presentation. Would targeting antigens to the $CD205^+$ DCs be more efficient at generating $CD8^+$ T cell responses? How do the mouse studies relate to the human immune system?

Targeting LCs for antigen delivery may be an optimal strategy for the induction of potent antigen-specific CTL responses. LC-specific molecule, such as Langerin, can be used as a target DC receptor (Idoyaga et al., 2008). Dermal $CD14^+$ DCs might represent the appropriate target for the induction of potent humoral responses (Figure 4). Selection of an appropriate adjuvant is also a critical parameter for the induction of the immunity of the desired type. For example, although TLR-ligands are widely considered to promote protective immunity against infectious agents, selecting the appropriate ligand will be critical. For instance, TLR2 ligation, which promotes the induction of Treg cells rather than Th1 or Th17 cells (Manicassamy et al., 2009), does not appear to be a preferred option for cancer vaccines. Thus, the challenge is to match the molecular target on DCs with the desired immune outcome, mimicking in many ways the natural role of these DC receptors to fine tune responses appropriate to the infection. Another strategy to target DCs is the usage of probiotic lactic acid bacteria to target mucosal DCs in the gut upon oral administration. Genetic manipulation of such bacteria could allow coupling of antigen expression and adjuvant effect of microbial products (Mohamadzadeh et al., 2008).

DCs originating from a specific tissue have the capacity to instruct T cells to home back to that tissue (Mora et al., 2003), and different DC subsets might provide even more detailed instructions. Furthermore, DCs activated by different adjuvants could induce T cells with entirely different migration properties. Addressing this aspect is critical for the design of vaccines, where optimal sites for T cell migration may vary in different disease states. For example, whereas vaccines against melanoma are expected to induce T cells that migrate into tumor sites including skin, vaccines against influenza virus are desired to induce T cells to migrate into airway mucosal surfaces. Therefore, multiple parameters need to be considered for the development of DC targeting vaccines. These include: (1) biological function of target DC subsets (induction of humoral versus cellular immunity), (2) the tissue distribution and receptors expressed by the target population to ensure antigen delivery, and (3) the activation receptors expressed by a given DC subset so as to guide the choice of adjuvant. Thus, a more complete understanding of the human DC subset biology will be necessary for the next generation of efficient DC-based vaccines. Other essential components of a successful vaccine are the selection of antigen and its formulation. These issues will be discussed below in the context of therapeutic cancer vaccines.

## Therapeutic Vaccines in Cancer

The prospect of DC-targeted vaccines for the treatment of infectious disease seems promising. Recently, active immunization against an infectious agent has been shown to hold great promise in cancer, namely the prevention of HPV-positive cervical cancer by vaccinating with a recombinant viral capsid protein (Harper

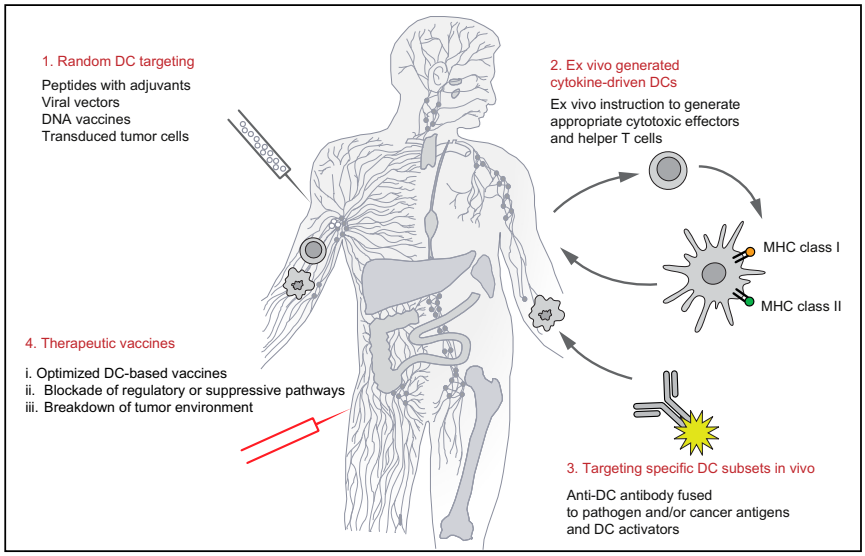


**Figure 5. Approaches to DC-Based Therapeutic Vaccination in Cancer and Chronic Infections**

(1) Vaccines based on antigen with or without adjuvant that target DCs randomly. That might result in vaccine antigens being taken up by a "wrong" type of DCs in the periphery, which might lead to "unwanted" type of immune response. Vaccine antigens could also flow to draining lymph nodes where they can be captured by resident DCs. (2) Vaccines based on ex vivo-generated antigen-loaded cytokine-driven DCs that are injected back into patients and (3) specific in vivo DC targeting with anti-DC antibodies linked (by fusion or conjugation) fused antigens and with DC activators. (4) Next generation clinical trials will test optimized DC vaccines combined with patient-adjusted approaches to block Treg cells and to break down the suppressive tumor environment. These therapies will be tested in preselected patients, thereby leading to personalized therapy.

et al., 2006). Unfortunately, the vast majority of human cancers do not have an obvious etiologic agent, so vaccine approaches in oncology would have to be therapeutic. In cancer, however, this task comes with a number of special challenges. First and foremost, most cancer antigens are nonmutated self-proteins and thus the repertoire is depleted of high-avidity clones through negative selection (Finn, 2003). As a result, tolerance must be overcome, and overcome in the context of patients whose tumors often induce a tolerogenic milieu.

Numerous approaches for the therapeutic vaccination of humans with cancer have been developed including autologous and allogeneic tumor cells (which are often modified to express various cytokines), peptides, proteins, and DNA vaccines (Figure 5; reviewed in Dougan and Dranoff, 2009). The observed results have been variable, yet in many cases, a tumor-specific immune response could be measured. The clinical efficacy of therapeutic vaccination in cancer has been questioned (Rosenberg et al., 2004) because of the limited rate of objective tumor regressions observed in clinical trials. At least two issues need to be considered: (1) the quality of immune responses that these early cancer vaccines were capable of eliciting and (2) definitions of clinical endpoints allowing assessment of efficacy.

Concerning the first point, the vast majority of early attempts at cancer vaccines were performed in the absence of any firm understanding of DCs or their role in immunization. The targeting of untargeted peptides, often in weak or ineffective adjuvants, was (and still is, even in some large ongoing clinical trials) commonplace. It should be clear that such approaches should have had, a priori, a low likelihood of even generating a robust immune response, much less one that is therapeutically protective.

Concerning the second point, defining clinical endpoints, the use of conventional RECIST (response evaluation criteria in solid tumors) measures to judge efficacy has been challenged by recent clinical trials testing anti-CTLA4 (ipilumimab) in patients with stage IV melanoma. There, in a randomized phase III clinical trial, a 2-fold improved overall survival in patients who received anti-CTLA4 was observed, but without early indications of tumor shrinkage (Hodi et al., 2010). In another indication an active immunotherapy product, sipuleucel-T (APC8015), based on the PBMCs activated with a fusion protein of prostate cancer antigen such as prostatic acid phosphatase PAP with GM-CSF, resulted in approximately 4 month-prolonged median survival in phase III trials in patients with prostate cancer (Higano et al., 2009). In both studies, the analysis of survival curves shows the separation only after 4–6 months, suggesting a certain delay in the treatment effect, just as one would expect if efficacy could occur only after the induction or redirection of antitumor immunity. Many questions remain concerning the therapeutic mechanisms underlying the results obtained in these trials. Yet, these studies will help define the basic principles of active immunotherapy that set this treatment modality apart from chemotherapy, radiotherapy, targeted therapies, and even adoptive T cell transfer.

Unlike what happens when conventional cytotoxic therapies are used, the time in which it takes to build tumor immunity tumors might progress before they actually regress, and tumors might appear clinically enlarged because of inflammation associated with active immune responses and lymphocyte infiltration. Thus, the clinical oncologist's and drug developer's expectation of instantaneous tumor "melting" may have to be managed, as may also be the case even for many ultimately effective non-immune-based targeted therapies. Although it may be tempting to conclude that overall survival may be the only true parameter of clinical efficacy, such a situation would greatly impede progress and patient access to new therapies because survival-based trials can be exceedingly long and costly. The need for modernized objective, quantifiable response criteria cannot be overemphasized. In this context, a number of studies demonstrated in small groups of patients with cancer that a success or failure of therapeutic vaccination is correlated with the expansion of antigen-specific effector T cells (Paczesny et al., 2004; Welters et al., 2010). Patients who fail are those in whom antigen-specific $CD4^+CD25^+Foxp3^+$ regulatory T cells outnumber the antigen-specific effector T cells (Welters et al., 2010). Thus, antigen-specific immune responses should remain among the key parameters of efficacy. A better understanding of how

effective vaccines, for example influenza vaccine or yellow fever vaccine, stimulate protective immune responses (Gaucher et al., 2008; Querec et al., 2009) might contribute to a better understanding of immune parameters of vaccine efficacy in cancer and chronic infections. Indeed, engineering vaccines to precisely target pathogens and cancer cells requires establishing the laws of immunity (Yewdell, 2010).

### Cell-Based Vaccines

Ex vivo-generated DCs have been used as therapeutic vaccines in patients with metastatic cancer for more than a decade and early studies have been reviewed elsewhere (Palucka et al., 2007). Importantly, a number of clinical studies have shown that DCs can expand T cells specific for nonmutated self proteins that are overexpressed in cancer. The analysis of immunological and clinical responses yields three patient groups: (1) one with no response, (2) one with immunological response but no clinical responses, and (3) one with both immunological and clinical responses. This third group is currently the smallest one but these patients are essential and they need to be studied in-depth because they will eventually permit us to understand the immune mechanisms that need to be established to control tumor growth and eliminate established tumors.

From the analysis of vaccinated patients, four parameters emerge as critical to understanding whether a vaccine-induced immune response can be protective: (1) the quality of elicited CTLs, (2) the quality of induced $CD4^+$ helper T cells, (3) the elimination and/or nonactivation of Treg cells, and (4) the breakdown of the immunosuppressive tumor microenvironment. Indeed, $CD8^+$ T cells play important roles in clearance of tumor cells and infected cells and are the actual drug elicited by vaccines. The immune responses elicited by the first-generation DC vaccines might not be of the quality required to allow the rejection of bulky tumors. For example, the induced $CD8^+$ T cells might not migrate into the tumor lesions (Appay et al., 2008; Harlin et al., 2009). Furthermore, low-avidity $CD8^+$ T cells might not be able to recognize peptide-MHC class I complexes on tumor cells and/or to kill them (Appay et al., 2008). Finally, the tumor microenvironment might inhibit effector $CD8^+$ T cell functions, for example, through myeloid-derived suppressor cells and Treg cells (for review see Gabrilovich and Nagaraj, 2009). In this context, the quality of $CD4^+$ T cells also represents a parameter essential for the outcome of immune response. $CD4^+$ T cells can contribute to antitumor immunity (Pardoll and Topalian, 1998) through different mechanisms including (1) provision of help in the expansion of tumor antigen-specific CTLs (Antony et al., 2005), (2) activation of macrophages at tumor sites (Corthay et al., 2005), (3) active killing of tumor cells (Quezada et al., 2010), and (4) the induction of long-term memory $CD8^+$ T cells (Sun and Bevan, 2003). However, $CD4^+$ T cells can also be detrimental, be it in the form of Treg cells that might dampen elicited $CD8^+$ T cell responses (Roncarolo et al., 2001a) or protumor type 2 cytokine-secreting $CD4^+$ T cells that counteract antitumor immunity by promoting tumor development (Aspord et al., 2007) and/or by polarizing tumor-associated macrophages (DeNardo et al., 2009).

The recent progress in immunomonitoring of specific immune responses in the blood (Palucka et al., 2006) and at the tumor site should help us address these questions. Modern approaches including polychromatic flow cytometry rather than the analysis of a single cytokine (e.g., IFN-$\gamma$ ELISPOT) and/or frequency of tetramer-positive cells will contribute to a better assessment of the quality of the immune responses elicited in the patients (Seder et al., 2008). Indeed, several studies, mostly performed in the context of HIV vaccines, have led to the conclusion that a mere measurement of the frequency of IFN-$\gamma$-secreting $CD8^+$ T cells is insufficient to evaluate the quality of vaccine-elicited immunity (Appay et al., 2008).

### Antibody-Based Vaccines

The experimental success of using DC-specific antibodies to target antigens to individual DC subsets in conjunction with appropriately chosen adjuvants has appealing potential for the design of anticancer vaccines. Combined with a powerful adjuvant, vaccinating with one or multiple tumor-derived antigens coupled to DC-specific antibodies may amplify existing responses or break tolerance, enabling the generation of protective responses. Because such responses would have to be MHC class I restricted, the approach might be more efficient if directed at DC populations adapted for cross-presentation, together with adjuvants that will activate their particular TLRs. Studies to date demonstrate the targeting of tumor antigens to DCs and LCs (Flacher et al., 2009) and the generation of therapeutic antitumor immunity (Sancho et al., 2008) in animal models. The $BDCA3^+$ subpopulation of myeloid DCs, as the likely human homolog of the $CD8\alpha^+$ DC subpopulation, may be of special interest with respect to their potential for priming $CD8^+$ T cell responses.

Furthermore, targeting both tumor and control antigens to human DCs ex vivo can lead to efficient antigen presentation and generation of $CD4^+$ T cell (Birkholz et al., 2010) and $CD8^+$ T cell (Bozzacco et al., 2007; Klechevsky et al., 2010) responses. Importantly, certain lectins, including Dectin-1, LOX-1, and DC-SIGN, as well as other DC surface molecules (e.g., CD40), also provide activation signals (Brown, 2006; Delneste et al., 2002; Figdor et al., 2002; Geijtenbeek et al., 2004). They can thus be exploited for both antigen delivery and activation pathway in a single targeted vaccine. The therapeutic success of these vaccines will build on the recent knowledge and progresses in our understanding of the biology of human DC subsets, cutaneous mDCs in particular.

A major challenge of this approach will be achieving not just T cell responses, but T cell responses that are sufficiently robust and long lasting so as to be clinically active. In the case of cancer, however, it will be possible to treat patients repeatedly and with more aggressive adjuvant combinations than is traditionally the case when developing prophylactic vaccines for infectious agents. In addition, it will almost certainly be beneficial to combine any such vaccination approaches with other agents, both immune and nonimmune, as discussed below.

Other antigen delivery systems are also under active investigation, particularly viral vector based. However, less is known regarding how such vectors enable antigen and adjuvant delivery to DCs.

### The Problem of Antigen Selection

Another major challenge remains the selection of antigen. The problem is relatively straight forward for prophylactic vaccines, assuming one understands which epitopes are neutralizing, expressed during human infection, and immunogenic. In the case of cancer antigens, the choice is less clear.

Candidate tumor antigens include (1) unique (mutated) antigens and (2) shared self-antigens (Parmiani et al., 2007). The choice between these types of antigens for vaccination could be viewed as choice between inducing immunity (mutated antigens, antigens not expressed during negative selection in the thymus) or breaking tolerance and inducing autoimmunity (overexpressed antigen, differentiation antigens). The debate about which type of antigen will be most effective is still open and will probably remain open until optimized delivery vehicles and adjuvants are developed for use in humans. The presumed advantages of mutated antigens are based on their potential to be recognized as non-self by the immune system and their potential resistance to negative selection in case the mutated protein is essential for cell survival, such as the B-Raf V600E epitope in melanoma (Parmiani et al., 2007). Furthermore, mutated antigens may select T cell receptors of higher affinity than shared antigens (resulting from the absence of thymic-negative selection) and minimize the prevalence of antigen-specific Treg cells (unless the tumor has already induced self-tolerance in the periphery) (Parmiani et al., 2007).

An example of a very potent antitumor and autoreactive response against self-antigen is provided by studies on paraneoplastic diseases and onconeuronal antigens (Darnell, 1994). Onconeural antigens that are normally expressed in immune privileged sites, such as neurons, can also be expressed in some cases of breast and ovarian cancer. In these patients a strong antigen-specific $CD8^+$ T cell response is generated (Albert et al., 1998), which provides effective tumor control but also autoreactive neurologic disease, paraneoplastic cerebellar degeneration. It is also the case that in melanoma patients, the existence of robust T cell responses to tumor-associated antigens (even shared antigens) is common (Nagorsen et al., 2003). Thus, immunity has occurred, it is just not protective, because of either T cell anergy or Treg cell prevalence. The example proves, however, that it is possible to generate T cell responses, even endogenously, to tumor antigens. Indeed, recent results have demonstrated that antigen-specific T cells accumulate within tumor beds in melanoma patients (Rosenberg and Dudley, 2009). A vaccine would try to amplify or redirect these responses to therapeutic efficacy.

Various groups have attempted to rank the potential of the numerous cancer-associated antigens that have been described to date (Cheever et al., 2009). In the absence of objective data in humans, it is difficult to make such assessments, so another approach has been to score either the expression of genes giving rise to tumor antigens or the physical presence of individual peptide-MHC class I complexes expressed at the surface of tumor cells. To obtain optimal coverage, even for a tumor in an individual patient, it may be necessary to immunize with several antigens simultaneously, although a single strong response may be sufficient. It is also possible that the best antigens will not be abundant as peptides at the tumor cell surface, and therefore not detectable by biochemical approaches. Absent approaches that enable the DC targeting of complex mixtures of tumor antigens, it seems most reasonable to begin this effort by using those antigens that can be objectively identified in the hope that improved delivery approaches and adjuvants will yield positive, protective immune responses. Focused preclinical and clinical studies should be employed to test this hypothesis.

Thus far, most focus has been placed on protein antigens whose peptides can be presented on the cell surface in complexes with classical MHC molecules (Townsend et al., 1985). However, tumors also express altered lipids and sugars that are presented by CD1 molecules (Hava et al., 2005). These can also be harnessed for improved vaccination, for example NKT cells that are thought to recognize lipid antigens can generate protective response with IFN-$\gamma$ secretion (Fujii et al., 2002). Accordingly, injection of cancer patients with DCs loaded with NKT cell ligand alpha-galactosyl-ceramide leads to sustained expansion of antigen-specific T cells (Chang et al., 2005).

A potentially interesting approach in the selection of antigen targets is suggested by the possibility that tumors are maintained by specialized subpopulations of "cancer stem cells" (Lobo et al., 2007). Although the definition and identity of these cells remains highly controversial, tumor cells that routinely survive conventional chemotherapy or targeted therapies are the ones that are responsible for tumor relapse and death. If these cells have special properties, or even if not, combining vaccine approaches with nonlymphoablative front line therapies may provide an optimal setting for generating protective immune responses.

### *Antigen Formulation*

An additional important problem is the form of antigen that should be delivered in the context of a vaccine, either preventative or therapeutic. Although peptides have often been used for immunization, as free entities, peptides have poor pharmacokinetic properties and are rapidly cleared. Coupling them to carriers helps somewhat, but chemical or genetic coupling to DC-targeted antibodies would appear the most efficient approach to get them to their required destinations. The use of peptides, of course, may presuppose the identification of relevant T cell epitopes, so conceivably the use of proteins may be preferable, or protein-peptide mixtures contained within an antibody-targeted carrier device (e.g., nanoparticle), which would enable the use of multiple potential antigens. In this context, recent studies indicate improved immunogenicity when viral antigens from HPV (Kenter et al., 2009) or HIV (Pialoux et al., 2001) are delivered in the form of long peptides together with adjuvants.

A further consideration is antigen stability. DCs exhibit a remarkably attenuated capacity for protein degradation, which serves to extend the longevity of internalized antigens, enabling a constant supply of endogenously produced peptides for loading on to both MHC class I and class II molecules (Delamarre et al., 2005). The simple rule, then, is that antigens (even endogenous ones) that are long lived are generally better than antigens that are more rapidly degraded (Delamarre et al., 2006). The extracellular and intracellular fates of antigens therefore will matter, and attention needs to be paid toward providing administered antigens in a form that maximizes half life.

## Are DCs Enough?

In view of the remarkable diversity of suppressive pathways present in patients with metastatic cancer, any durable clinical response elicited by vaccination is already an achievement. However, to improve the outcomes, DC vaccines need to be combined, in particular for patients at advanced stages, with other therapies that offset the suppressive tumor environment

(Dougan and Dranoff, 2009; Melief, 2008). Such combination regimens will involve several intervention strategies that target different pathways. In particular, blocking antibodies or soluble receptors can be exploited for the blockade of suppressive cytokines in the tumor microenvironment, for example IL-10 (Moore et al., 2001), IL-13 (Terabe et al., 2000), or TGF-β (Li et al., 2006). Tumor cells can often express surface molecules that inherently suppress T cell activity, notably PD-L1, which comes up especially in tumors that express oncogenic mutations in the PI3-kinase pathway. Antibodies to PD-1 on activated T cells, or to PD-L1, might thus reverse T cell exhaustion or anergy (Pilon-Thomas et al., 2010), and in early clinical studies, treatment with anti-PD1 exerts some beneficial clinical effect (Brahmer et al., 2010). It is a common observation in melanoma (and other cancers) that patients exhibit pre-existing T cell responses without a vaccine ever having been purposefully administered. These T cells are not protective, or at least not sufficiently protective, despite the fact that they can often be recovered from tumor beds (Rosenberg and Dudley, 2009), suggesting that reactivation strategies may be useful on their own.

These examples emphasize that DCs may not be enough, and in some cases, may not even be strictly necessary, at least from the treating physician's point of view. An endogenous vaccine may be created by necrotic or apoptotic death of tumor cells after chemotherapy or targeted therapy, where tumor antigens released in conjunction with "danger signals" from the dying cells are internalized by infiltrating DCs and then presented to T cells. A further therapeutic vaccine may help amplify these responses, or perhaps retool them to be more immunoprotective than immunoregulatory, but a more effective approach in such instances might be to target the T cells themselves. This is the goal of anti-CTLA4-based therapies (Peggs et al., 2009). Conceivably, antibodies to PD1-PDL1 might also achieve this goal, and in a fashion with less autoimmune toxicity, because only those T cells encountering their cognate antigen in the context of PD1-PDL1 interactions would be stimulated.

Just as different tumors are currently treated with different combinations of cytostatic drugs and targeted therapies, we foresee the development of clinical protocols combining DC vaccines with individualized adjunct therapies, most probably those involving nonlymphoablative cytotoxic or targeted therapies (Figure 5). In melanoma, the recent demonstration of dramatic but transient responses in patients expressing the V600E oncogenic mutation with a specific B-Raf inhibitor (Boni et al., 2010) creates a remarkable opportunity to implement just such combination therapies. For such complex therapies to be designed rationally, however, careful attention will have to be paid to profiling the immunological status of individual patients and their tumors before, during, and after therapy. Patient selection and immunological markers attesting to the effects of a given therapeutic attempt will be key to understanding why an approach does or does not work.

## We Have a Dream

Studies performed in the last decade have highlighted the commonalities and uniqueness of the various DC subsets. This new knowledge represents a fertile ground to work on to design better strategies for intervening in numerous clinical situations. The capacity of LCs and $CD14^+$ DCs to preferentially prime cellular immunity and humoral immunity, respectively, has significant implications, most particularly in the context of novel human vaccines. Thus, targeting LCs will be important for the design of vaccines that aim at eliciting strong cellular immunity. Such vaccines might be particularly useful at preventing, and perhaps even treating, chronic diseases including viral (HIV, hepatitis C virus), bacterial (mycobacteria), and parasitic (malaria) diseases, as well as cancer. The most efficient vaccines might actually be those that will target both $CD14^+$ DCs and LCs, thereby allowing the maximal stimulation of both humoral and cellular immune responses. In this regard it is intriguing to consider that one of the most effective vaccines, smallpox vaccine, acts through a combination of strong cellular and humoral immunity and requires scarification of the skin, a procedure that injures both epidermis and dermis and that is likely to mobilize and activate LCs as well as dermal DCs. Likewise, one of the most potent vaccines ever generated against yellow fever (YF17D) activates multiple DC subsets (Querec et al., 2006) and leads to integrated immune response that includes both humoral and cellular immunity (Gaucher et al., 2008).

We foresee that the improved vaccines that target DCs will permit us to treat and prevent many chronic diseases, and likewise, manipulation of DCs will also permit us to dampen overly enhanced immune responses as occurs in allergy and autoimmunity possibly by turning on regulatory mechanisms.

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## REFERENCES

Albert, M.L., Darnell, J.C., Bender, A., Francisco, L.M., Bhardwaj, N., and Darnell, R.B. (1998). Tumor-specific killer cells in paraneoplastic cerebellar degeneration. Nat. Med. *4*, 1321–1324.

Allan, R.S., Smith, C.M., Belz, G.T., van Lint, A.L., Wakim, L.M., Heath, W.R., and Carbone, F.R. (2003). Epidermal viral immunity induced by CD8alpha+ dendritic cells but not by Langerhans cells. Science *301*, 1925–1928.

Alvarez, D., Harder, G., Fattouh, R., Sun, J., Goncharova, S., Stämpfli, M.R., Coyle, A.J., Bramson, J.L., and Jordana, M. (2005). Cutaneous antigen priming via gene gun leads to skin-selective Th2 immune-inflammatory responses. J. Immunol. *174*, 1664–1674.

Antony, P.A., Piccirillo, C.A., Akpinarli, A., Finkelstein, S.E., Speiss, P.J., Surman, D.R., Palmer, D.C., Chan, C.C., Klebanoff, C.A., Overwijk, W.W., et al. (2005). $CD8^+$ T cell immunity against a tumor/self-antigen is augmented by $CD4^+$ T helper cells and hindered by naturally occurring T regulatory cells. J. Immunol. *174*, 2591–2601.

Appay, V., Douek, D.C., and Price, D.A. (2008). CD8+ T cell efficacy in vaccination and disease. Nat. Med. *14*, 623–628.

Arima, K., Watanabe, N., Hanabuchi, S., Chang, M., Sun, S.C., and Liu, Y.J. (2010). Distinct signal codes generate dendritic cell functional plasticity. Sci. Signal. *3*, ra4.

Aspord, C., Pedroza-Gonzalez, A., Gallegos, M., Tindle, S., Burton, E.C., Su, D., Marches, F., Banchereau, J., and Palucka, A.K. (2007). Breast cancer instructs dendritic cells to prime interleukin 13-secreting CD4+ T cells that facilitate tumor development. J. Exp. Med. *204*, 1037–1047.

Bachem, A., Güttler, S., Hartung, E., Ebstein, F., Schaefer, M., Tannert, A., Salama, A., Movassaghi, K., Opitz, C., Mages, H.W., et al. (2010). Superior antigen cross-presentation and XCR1 expression define human CD11c+CD141+ cells as homologues of mouse CD8+ dendritic cells. J. Exp. Med. *207*, 1273–1281.

Bedoui, S., Whitney, P.G., Waithman, J., Eidsmo, L., Wakim, L., Caminschi, I., Allan, R.S., Wojtasiak, M., Shortman, K., Carbone, F.R., et al. (2009). Cross-presentation of viral and self antigens by skin-derived CD103+ dendritic cells. Nat. Immunol. *10*, 488–495.

Birkholz, K., Schwenkert, M., Kellner, C., Gross, S., Fey, G., Schuler-Thurner, B., Schuler, G., Schaft, N., and Dörrie, J. (2010). Targeting of DEC-205 on human dendritic cells results in efficient MHC class II-restricted antigen presentation. Blood *116*, 2277–2285.

Bluestone, J.A., Mackay, C.R., O'Shea, J.J., and Stockinger, B. (2009). The functional plasticity of T cell subsets. Nat. Rev. Immunol. *9*, 811–816.

Boni, A., Cogdill, A.P., Dang, P., Udayakumar, D., Njauw, C.N., Sloss, C.M., Ferrone, C.R., Flaherty, K.T., Lawrence, D.P., Fisher, D.E., et al. (2010). Selective BRAFV600E inhibition enhances T-cell recognition of melanoma without affecting lymphocyte function. Cancer Res. *70*, 5213–5219.

Bonifaz, L., Bonnyay, D., Mahnke, K., Rivera, M., Nussenzweig, M.C., and Steinman, R.M. (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.

Bozzacco, L., Trumpfheller, C., Siegal, F.P., Mehandru, S., Markowitz, M., Carrington, M., Nussenzweig, M.C., Piperno, A.G., and Steinman, R.M. (2007). DEC-205 receptor on dendritic cells mediates presentation of HIV gag protein to CD8+ T cells in a spectrum of human MHC I haplotypes. Proc. Natl. Acad. Sci. USA *104*, 1289–1294.

Brahmer, J.R., Drake, C.G., Wollner, I., Powderly, J.D., Picus, J., Sharfman, W.H., Stankevich, E., Pons, A., Salay, T.M., McMiller, T.L., et al. (2010). Phase I study of single-agent anti-programmed death-1 (MDX-1106) in refractory solid tumors: Safety, clinical activity, pharmacodynamics, and immunologic correlates. J. Clin. Oncol. *28*, 3167–3175.

Brown, G.D. (2006). Dectin-1: A signalling non-TLR pattern-recognition receptor. Nat. Rev. Immunol. *6*, 33–43.

Brown, G.D. (2008). Sensing necrosis with Mincle. Nat. Immunol. *9*, 1099–1100.

Cao, W., Rosen, D.B., Ito, T., Bover, L., Bao, M., Watanabe, G., Yao, Z., Zhang, L., Lanier, L.L., and Liu, Y.J. (2006). Plasmacytoid dendritic cell-specific receptor ILT7-Fc epsilonRI gamma inhibits Toll-like receptor-induced interferon production. J. Exp. Med. *203*, 1399–1405.

Celluzzi, C.M., and Falo, L.D., Jr. (1997). Epidermal dendritic cells induce potent antigen-specific CTL-mediated immunity. J. Invest. Dermatol. *108*, 716–720.

Chang, D.H., Osman, K., Connolly, J., Kukreja, A., Krasovsky, J., Pack, M., Hutchinson, A., Geller, M., Liu, N., Annable, R., et al. (2005). Sustained expansion of NKT cells and antigen-specific T cells after injection of alpha-galactosyl-ceramide loaded mature dendritic cells in cancer patients. J. Exp. Med. *201*, 1503–1517.

Cheever, M.A., Allison, J.P., Ferris, A.S., Finn, O.J., Hastings, B.M., Hecht, T.T., Mellman, I., Prindiville, S.A., Viner, J.L., Weiner, L.M., and Matrisian, L.M. (2009). The prioritization of cancer antigens: a national cancer institute pilot project for the acceleration of translational research. Clin. Cancer Res. *15*, 5323–5337.

Chen, L. (2004). Co-inhibitory molecules of the B7-CD28 family in the control of T-cell immunity. Nat. Rev. Immunol. *4*, 336–347.

Cheng, P., Zhou, J., and Gabrilovich, D. (2010). Regulation of dendritic cell differentiation and function by Notch and Wnt pathways. Immunol. Rev. *234*, 105–119.

Corthay, A., Skovseth, D.K., Lundin, K.U., Røsjø, E., Omholt, H., Hofgaard, P.O., Haraldsen, G., and Bogen, B. (2005). Primary antitumor immune response mediated by $CD4^+$ T cells. Immunity *22*, 371–383.

Crozat, K., Guiton, R., Contreras, V., Feuillet, V., Dutertre, C.A., Ventre, E., Vu Manh, T.P., Baranek, T., Storset, A.K., Marvel, J., et al. (2010). The XC chemokine receptor 1 is a conserved selective marker of mammalian cells homologous to mouse CD8alpha+ dendritic cells. J. Exp. Med. *207*, 1283–1292.

Darnell, R.B. (1994). Paraneoplastic syndromes. In Current Diagnosis in Neurobiology, E. Feldmann, ed. (Philadelphia, PA: Mosbey-Year Book), pp. 137–141.

Delamarre, L., Pack, M., Chang, H., Mellman, I., and Trombetta, E.S. (2005). Differential lysosomal proteolysis in antigen-presenting cells determines antigen fate. Science *307*, 1630–1634.

Delamarre, L., Couture, R., Mellman, I., and Trombetta, E.S. (2006). Enhancing immunogenicity by limiting susceptibility to lysosomal proteolysis. J. Exp. Med. *203*, 2049–2055.

Delneste, Y., Magistrelli, G., Gauchat, J., Haeuw, J., Aubry, J., Nakamura, K., Kawakami-Honda, N., Goetsch, L., Sawamura, T., Bonnefoy, J., and Jeannin, P. (2002). Involvement of LOX-1 in dendritic cell-mediated antigen cross-presentation. Immunity *17*, 353–362.

DeNardo, D.G., Barreto, J.B., Andreu, P., Vasquez, L., Tawfik, D., Kolhatkar, N., and Coussens, L.M. (2009). CD4(+) T cells regulate pulmonary metastasis of mammary carcinomas by enhancing protumor properties of macrophages. Cancer Cell *16*, 91–102.

Di Pucchio, T., Chatterjee, B., Smed-Sörensen, A., Clayton, S., Palazzo, A., Montes, M., Xue, Y., Mellman, I., Banchereau, J., and Connolly, J.E. (2008). Direct proteasome-independent cross-presentation of viral antigen by plasmacytoid dendritic cells on major histocompatibility complex class I. Nat. Immunol. *9*, 551–557.

Domínguez, P.M., and Ardavín, C. (2010). Differentiation and function of mouse monocyte-derived dendritic cells in steady state and inflammation. Immunol. Rev. *234*, 90–104.

Dougan, M., and Dranoff, G. (2009). Immune therapy for cancer. Annu. Rev. Immunol. *27*, 83–117.

Dudziak, D., Kamphorst, A.O., Heidkamp, G.F., Buchholz, V.R., Trumpfheller, C., Yamazaki, S., Cheong, C., Liu, K., Lee, H.W., Park, C.G., et al. (2007). Differential antigen processing by dendritic cell subsets in vivo. Science *315*, 107–111.

Dullaers, M., Li, D., Xue, Y., Ni, L., Gayet, I., Morita, R., Ueno, H., Palucka, K.A., Banchereau, J., and Oh, S. (2009). A T cell-dependent mechanism for the induction of human mucosal homing immunoglobulin A-secreting plasmablasts. Immunity *30*, 120–129.

Dzionek, A., Fuchs, A., Schmidt, P., Cremer, S., Zysk, M., Miltenyi, S., Buck, D.W., and Schmitz, J. (2000). BDCA-2, BDCA-3, and BDCA-4: Three markers for distinct subsets of dendritic cells in human peripheral blood. J. Immunol. *165*, 6037–6046.

Figdor, C.G., van Kooyk, Y., and Adema, G.J. (2002). C-type lectin receptors on dendritic cells and Langerhans cells. Nat. Rev. Immunol. *2*, 77–84.

Finn, O.J. (2003). Cancer vaccines: Between the idea and the reality. Nat. Rev. Immunol. *3*, 630–641.

Flacher, V., Sparber, F., Tripp, C.H., Romani, N., and Stoitzner, P. (2009). Targeting of epidermal Langerhans cells with antigenic proteins: Attempts to harness their properties for immunotherapy. Cancer Immunol. Immunother. *58*, 1137–1147.

Flornes, L.M., Bryceson, Y.T., Spurkland, A., Lorentzen, J.C., Dissen, E., and Fossum, S. (2004). Identification of lectin-like receptors expressed by antigen presenting cells and neutrophils and their mapping to a novel gene complex. Immunogenetics *56*, 506–517.

Fujii, S., Shimizu, K., Kronenberg, M., and Steinman, R.M. (2002). Prolonged IFN-gamma-producing NKT response induced with alpha-galactosylceramide-loaded DCs. Nat. Immunol. *3*, 867–874.

Fukaura, H., Kent, S.C., Pietrusewicz, M.J., Khoury, S.J., Weiner, H.L., and Hafler, D.A. (1996). Induction of circulating myelin basic protein and proteolipid protein-specific transforming growth factor-b1-secreting Th3 T cells by oral administration of myelin in multiple sclerosis patients. J. Clin. Invest. *98*, 70–77.

Gabrilovich, D.I., and Nagaraj, S. (2009). Myeloid-derived suppressor cells as regulators of the immune system. Nat. Rev. Immunol. *9*, 162–174.

Gaucher, D., Therrien, R., Kettaf, N., Angermann, B.R., Boucher, G., Filali-Mouhim, A., Moser, J.M., Mehta, R.S., Drake, D.R., 3rd, Castro, E., et al. (2008). Yellow fever vaccine induces integrated multilineage and polyfunctional immune responses. J. Exp. Med. *205*, 3119–3131.

Geijtenbeek, T.B., van Vliet, S.J., Engering, A., 't Hart, B.A., and van Kooyk, Y. (2004). Self- and nonself-recognition by C-type lectins on dendritic cells. Annu. Rev. Immunol. *22*, 33–54.

Geissmann, F., Gordon, S., Hume, D.A., Mowat, A.M., and Randolph, G.J. (2010). Unravelling mononuclear phagocyte heterogeneity. Nat. Rev. Immunol. *10*, 453–460.

Greenwald, R.J., Freeman, G.J., and Sharpe, A.H. (2005). The B7 family revisited. Annu. Rev. Immunol. *23*, 515–548.

Harlin, H., Meng, Y., Peterson, A.C., Zha, Y., Tretiakova, M., Slingluff, C., McKee, M., and Gajewski, T.F. (2009). Chemokine expression in melanoma metastases associated with CD8+ T-cell recruitment. Cancer Res. *69*, 3077–3085.

Harper, D.M., Franco, E.L., Wheeler, C.M., Moscicki, A.B., Romanowski, B., Roteli-Martins, C.M., Jenkins, D., Schuind, A., Costa Clemens, S.A., and Dubin, G.; HPV Vaccine Study group. (2006). Sustained efficacy up to 4.5 years of a bivalent L1 virus-like particle vaccine against human papillomavirus types 16 and 18: follow-up from a randomised control trial. Lancet *367*, 1247–1255.

Hava, D.L., Brigl, M., van den Elzen, P., Zajonc, D.M., Wilson, I.A., and Brenner, M.B. (2005). CD1 assembly and the formation of CD1-antigen complexes. Curr. Opin. Immunol. *17*, 88–94.

Hawiger, D., Inaba, K., Dorsett, Y., Guo, M., Mahnke, K., Rivera, M., Ravetch, J.V., Steinman, R.M., and Nussenzweig, M.C. (2001). Dendritic cells induce peripheral T cell unresponsiveness under steady state conditions in vivo. J. Exp. Med. *194*, 769–779.

Hermans, I.F., Silk, J.D., Gileadi, U., Salio, M., Mathew, B., Ritter, G., Schmidt, R., Harris, A.L., Old, L., and Cerundolo, V. (2003). NKT cells enhance CD4+ and CD8+ T cell responses to soluble antigen in vivo through direct interaction with dendritic cells. J. Immunol. *171*, 5140–5147.

Higano, C.S., Schellhammer, P.F., Small, E.J., Burch, P.A., Nemunaitis, J., Yuh, L., Provost, N., and Frohlich, M.W. (2009). Integrated data from 2 randomized, double-blind, placebo-controlled, phase 3 trials of active cellular immunotherapy with sipuleucel-T in advanced prostate cancer. Cancer *115*, 3670–3679.

Hodi, F.S., O'Day, S.J., McDermott, D.F., Weber, R.W., Sosman, J.A., Haanen, J.B., Gonzalez, R., Robert, C., Schadendorf, D., Hassel, J.C., et al. (2010). Improved survival with ipilimumab in patients with metastatic melanoma. N. Engl. J. Med. *363*, 711–723.

Hutloff, A., Dittrich, A.M., Beier, K.C., Eljaschewitsch, B., Kraft, R., Anagnostopoulos, I., and Kroczek, R.A. (1999). ICOS is an inducible T-cell co-stimulator structurally and functionally related to CD28. Nature *397*, 263–266.

Idoyaga, J., Cheong, C., Suda, K., Suda, N., Kim, J.Y., Lee, H., Park, C.G., and Steinman, R.M. (2008). Cutting edge: Langerin/CD207 receptor on dendritic cells mediates efficient antigen presentation on MHC I and II products in vivo. J. Immunol. *180*, 3647–3650.

Ito, T., Wang, Y.H., Duramad, O., Hanabuchi, S., Perng, O.A., Gilliet, M., Qin, F.X., and Liu, Y.J. (2006). OX40 ligand shuts down IL-10-producing regulatory T cells. Proc. Natl. Acad. Sci. USA *103*, 13138–13143.

Ito, T., Yang, M., Wang, Y.H., Lande, R., Gregorio, J., Perng, O.A., Qin, X.F., Liu, Y.J., and Gilliet, M. (2007). Plasmacytoid dendritic cells prime IL-10-producing T regulatory cells by inducible costimulator ligand. J. Exp. Med. *204*, 105–115.

Janssen, E.M., Lemmens, E.E., Wolfe, T., Christen, U., von Herrath, M.G., and Schoenberger, S.P. (2003). CD4+ T cells are required for secondary expansion and memory in CD8+ T lymphocytes. Nature *421*, 852–856.

Jego, G., Palucka, A.K., Blanck, J.P., Chalouni, C., Pascual, V., and Banchereau, J. (2003). Plasmacytoid dendritic cells induce plasma cell differentiation through type I interferon and interleukin 6. Immunity *19*, 225–234.

Jiang, A., Bloom, O., Ono, S., Cui, W., Unternaehrer, J., Jiang, S., Whitney, J.A., Connolly, J., Banchereau, J., and Mellman, I. (2007). Disruption of E-cadherin-mediated adhesion induces a functionally distinct pathway of dendritic cell maturation. Immunity *27*, 610–624.

Joffre, O., Nolte, M.A., Spörri, R., and Reis e Sousa, C. (2009). Inflammatory signals in dendritic cell activation and the induction of adaptive immunity. Immunol. Rev. *227*, 234–247.

Jongbloed, S.L., Kassianos, A.J., McDonald, K.J., Clark, G.J., Ju, X., Angel, C.E., Chen, C.J., Dunbar, P.R., Wadley, R.B., Jeet, V., et al. (2010). Human CD141+ (BDCA-3)+ dendritic cells (DCs) represent a unique myeloid DC subset that cross-presents necrotic cell antigens. J. Exp. Med. *207*, 1247–1260.

Kastelein, R.A., Hunter, C.A., and Cua, D.J. (2007). Discovery and biology of IL-23 and IL-27: Related but functionally distinct regulators of inflammation. Annu. Rev. Immunol. *25*, 221–242.

Kenter, G.G., Welters, M.J., Valentijn, A.R., Lowik, M.J., Berends-van der Meer, D.M., Vloon, A.P., Essahsah, F., Fathers, L.M., Offringa, R., Drijfhout, J.W., et al. (2009). Vaccination against HPV-16 oncoproteins for vulvar intraepithelial neoplasia. N. Engl. J. Med. *361*, 1838–1847.

Kissenpfennig, A., Henri, S., Dubois, B., Laplace-Builhé, C., Perrin, P., Romani, N., Tripp, C.H., Douillard, P., Leserman, L., Kaiserlian, D., et al. (2005). Dynamics and function of Langerhans cells in vivo: Dermal dendritic cells colonize lymph node areas distinct from slower migrating Langerhans cells. Immunity *22*, 643–654.

Klechevsky, E., Morita, R., Liu, M., Cao, Y., Coquery, S., Thompson-Snipes, L., Briere, F., Chaussabel, D., Zurawski, G., Palucka, A.K., et al. (2008). Functional specializations of human epidermal Langerhans cells and CD14+ dermal dendritic cells. Immunity *29*, 497–510.

Klechevsky, E., Liu, M., Morita, R., Banchereau, R., Thompson-Snipes, L., Palucka, A.K., Ueno, H., and Banchereau, J. (2009). Understanding human myeloid dendritic cell subsets for the rational design of novel vaccines. Hum. Immunol. *70*, 281–288.

Klechevsky, E., Flamar, A.L., Cao, Y., Blanck, J.P., Liu, M., O'Bar, A., Agouna-Deciat, O., Klucar, P., Thompson-Snipes, L., Zurawski, S., et al. (2010). Cross-priming CD8+ T cells by targeting antigens to human dendritic cells through DCIR. Blood *116*, 1685–1697.

Krummel, M.F., and Allison, J.P. (1995). CD28 and CTLA-4 have opposing effects on the response of T cells to stimulation. J. Exp. Med. *182*, 459–465.

Lanzavecchia, A., and Sallusto, F. (2001). Regulation of T cell immunity by dendritic cells. Cell *106*, 263–266.

Leslie, D.S., Vincent, M.S., Spada, F.M., Das, H., Sugita, M., Morita, C.T., and Brenner, M.B. (2002). CD1-mediated $\gamma/\delta$ T cell maturation of dendritic cells. J. Exp. Med. *196*, 1575–1584.

Levings, M.K., Gregori, S., Tresoldi, E., Cazzaniga, S., Bonini, C., and Roncarolo, M.G. (2005). Differentiation of Tr1 cells by immature dendritic cells requires IL-10 but not CD25+CD4+ Tr cells. Blood *105*, 1162–1169.

Li, M.O., Wan, Y.Y., Sanjabi, S., Robertson, A.K., and Flavell, R.A. (2006). Transforming growth factor-beta regulation of immune responses. Annu. Rev. Immunol. *24*, 99–146.

Liu, Y.J. (2005). IPC: Professional type 1 interferon-producing cells and plasmacytoid dendritic cell precursors. Annu. Rev. Immunol. *23*, 275–306.

Lobo, N.A., Shimono, Y., Qian, D., and Clarke, M.F. (2007). The biology of cancer stem cells. Annu. Rev. Cell Dev. Biol. *23*, 675–699.

Lucas, M., Schachterle, W., Oberle, K., Aichele, P., and Diefenbach, A. (2007). Dendritic cells prime natural killer cells by trans-presenting interleukin 15. Immunity *26*, 503–517.

Macatonia, S.E., Hosken, N.A., Litton, M., Vieira, P., Hsieh, C.-S., Culpepper, J.A., Wysocka, M., Trinchieri, G., Murphy, K.M., and O'Garra, A. (1995). Dendritic cells produce IL-12 and direct the development of Th1 cells from naive $CD4^+$ T cells. J. Immunol. *154*, 5071–5079.

Maldonado-López, R., De Smedt, T., Michel, P., Godfroid, J., Pajak, B., Heirman, C., Thielemans, K., Leo, O., Urbain, J., and Moser, M. (1999). $CD8\alpha^+$ and $CD8\alpha^-$ subclasses of dendritic cells direct the development of distinct T helper cells in vivo. J. Exp. Med. *189*, 587–592.

Manicassamy, S., and Pulendran, B. (2009). Modulation of adaptive immunity with Toll-like receptors. Semin. Immunol. *21*, 185–193.

Manicassamy, S., Ravindran, R., Deng, J., Oluoch, H., Denning, T.L., Kasturi, S.P., Rosenthal, K.M., Evavold, B.D., and Pulendran, B. (2009). Toll-like

receptor 2-dependent induction of vitamin A-metabolizing enzymes in dendritic cells promotes T regulatory responses and inhibits autoimmunity. Nat. Med. *15*, 401–409.

Manicassamy, S., Reizis, B., Ravindran, R., Nakaya, H., Salazar-Gonzalez, R.M., Wang, Y.C., and Pulendran, B. (2010). Activation of beta-catenin in dendritic cells regulates immunity versus tolerance in the intestine. Science *329*, 849–853.

Matsui, T., Connolly, J.E., Michnevitz, M., Chaussabel, D., Yu, C.I., Glaser, C., Tindle, S., Pypaert, M., Freitas, H., Piqueras, B., et al. (2009). CD2 distinguishes two subsets of human plasmacytoid dendritic cells with distinct phenotype and functions. J. Immunol. *182*, 6815–6823.

McCurley, N., and Mellman, I. (2010). Monocyte-derived dendritic cells exhibit increased levels of lysosomal proteolysis as compared to other human dendritic cell populations. PLoS ONE *5*, e11949.

Melief, C.J. (2008). Cancer immunotherapy by dendritic cells. Immunity *29*, 372–383.

Merad, M., Ginhoux, F., and Collin, M. (2008). Origin, homeostasis and function of Langerhans cells and other langerin-expressing dendritic cells. Nat. Rev. Immunol. *8*, 935–947.

Mestas, J., and Hughes, C.C.W. (2004). Of mice and not men: Differences between mouse and human immunology. J. Immunol. *172*, 2731–2738.

Mohamadzadeh, M., Berard, F., Essert, G., Chalouni, C., Pulendran, B., Davoust, J., Bridges, G., Palucka, A.K., and Banchereau, J. (2001). Interleukin 15 skews monocyte differentiation into dendritic cells with features of Langerhans cells. J. Exp. Med. *194*, 1013–1020.

Mohamadzadeh, M., Duong, T., Hoover, T., and Klaenhammer, T.R. (2008). Targeting mucosal dendritic cells with microbial antigens from probiotic lactic acid bacteria. Expert Rev. Vaccines *7*, 163–174.

Moore, K.W., de Waal Malefyt, R., Coffman, R.L., and O'Garra, A. (2001). Interleukin-10 and the interleukin-10 receptor. Annu. Rev. Immunol. *19*, 683–765.

Mora, J.R., Bono, M.R., Manjunath, N., Weninger, W., Cavanagh, L.L., Rosemblatt, M., and Von Andrian, U.H. (2003). Selective imprinting of gut-homing T cells by Peyer's patch dendritic cells. Nature *424*, 88–93.

Mosmann, T.R., Cherwinski, H., Bond, M.W., Giedlin, M.A., and Coffman, R.L. (1986). Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. J. Immunol. *136*, 2348–2357.

Münz, C., Steinman, R.M., and Fujii, S. (2005). Dendritic cell maturation by innate lymphocytes: Coordinated stimulation of innate and adaptive immunity. J. Exp. Med. *202*, 203–207.

Nagorsen, D., Scheibenbogen, C., Marincola, F.M., Letsch, A., and Keilholz, U. (2003). Natural T cell immunity against cancer. Clin. Cancer Res. *9*, 4296–4303.

Paczesny, S., Banchereau, J., Wittkowski, K.M., Saracino, G., Fay, J., and Palucka, A.K. (2004). Expansion of melanoma-specific cytolytic CD8+ T cell precursors in patients with metastatic melanoma vaccinated with CD34+ progenitor-derived dendritic cells. J. Exp. Med. *199*, 1503–1511.

Palucka, A.K., Ueno, H., Connolly, J., Kerneis-Norvell, F., Blanck, J.P., Johnston, D.A., Fay, J., and Banchereau, J. (2006). Dendritic cells loaded with killed allogeneic melanoma cells can induce objective clinical responses and MART-1 specific CD8+ T-cell immunity. J. Immunother. *29*, 545–557.

Palucka, A.K., Ueno, H., Fay, J.W., and Banchereau, J. (2007). Taming cancer by inducing immunity via dendritic cells. Immunol. Rev. *220*, 129–150.

Paquette, R.L., Hsu, N.C., Kiertscher, S.M., Park, A.N., Tran, L., Roth, M.D., and Glaspy, J.A. (1998). Interferon-alpha and granulocyte-macrophage colony-stimulating factor differentiate peripheral blood monocytes into potent antigen-presenting cells. J. Leukoc. Biol. *64*, 358–367.

Pardoll, D.M., and Topalian, S.L. (1998). The role of CD4+ T cell responses in antitumor immunity. Curr. Opin. Immunol. *10*, 588–594.

Parmiani, G., De Filippo, A., Novellino, L., and Castelli, C. (2007). Unique human tumor antigens: Immunobiology and use in clinical trials. J. Immunol. *178*, 1975–1979.

Peggs, K.S., Quezada, S.A., Chambers, C.A., Korman, A.J., and Allison, J.P. (2009). Blockade of CTLA-4 on both effector and regulatory T cell compartments contributes to the antitumor activity of anti-CTLA-4 antibodies. J. Exp. Med. *206*, 1717–1725.

Penna, G., and Adorini, L. (2000). 1 Alpha,25-dihydroxyvitamin D3 inhibits differentiation, maturation, activation, and survival of dendritic cells leading to impaired alloreactive T cell activation. J. Immunol. *164*, 2405–2411.

Pialoux, G., Gahéry-Ségard, H., Sermet, S., Poncelet, H., Fournier, S., Gérard, L., Tartar, A., Gras-Masse, H., Levy, J.P., and Guillet, J.G.; ANRS VAC 04 Study Team. (2001). Lipopeptides induce cell-mediated anti-HIV immune responses in seronegative volunteers. AIDS *15*, 1239–1249.

Pilon-Thomas, S., Mackay, A., Vohra, N., and Mulé, J.J. (2010). Blockade of programmed death ligand 1 enhances the therapeutic efficacy of combination immunotherapy against melanoma. J. Immunol. *184*, 3442–3449.

Poulin, L.F., Salio, M., Griessinger, E., Anjos-Afonso, F., Craciun, L., Chen, J.L., Keller, A.M., Joffre, O., Zelenay, S., Nye, E., et al. (2010). Characterization of human DNGR-1+ BDCA3+ leukocytes as putative equivalents of mouse CD8alpha+ dendritic cells. J. Exp. Med. *207*, 1261–1271.

Querec, T., Bennouna, S., Alkan, S., Laouar, Y., Gorden, K., Flavell, R., Akira, S., Ahmed, R., and Pulendran, B. (2006). Yellow fever vaccine YF-17D activates multiple dendritic cell subsets via TLR2, 7, 8, and 9 to stimulate polyvalent immunity. J. Exp. Med. *203*, 413–424.

Querec, T.D., Akondy, R.S., Lee, E.K., Cao, W., Nakaya, H.I., Teuwen, D., Pirani, A., Gernert, K., Deng, J., Marzolf, B., et al. (2009). Systems biology approach predicts immunogenicity of the yellow fever vaccine in humans. Nat. Immunol. *10*, 116–125.

Quezada, S.A., Simpson, T.R., Peggs, K.S., Merghoub, T., Vider, J., Fan, X., Blasberg, R., Yagita, H., Muranski, P., Antony, P.A., et al. (2010). Tumor-reactive CD4(+) T cells develop cytotoxic activity and eradicate large established melanoma after transfer into lymphopenic hosts. J. Exp. Med. *207*, 637–650.

Reis e Sousa, C. (2006). Dendritic cells in a mature age. Nat. Rev. Immunol. *6*, 476–483.

Romani, N., Gruner, S., Brang, D., Kämpgen, E., Lenz, A., Trockenbacher, B., Konwalinka, G., Fritsch, P.O., Steinman, R.M., and Schuler, G. (1994). Proliferating dendritic cell progenitors in human blood. J. Exp. Med. *180*, 83–93.

Roncarolo, M.G., Bacchetta, R., Bordignon, C., Narula, S., and Levings, M.K. (2001a). Type 1 T regulatory cells. Immunol. Rev. *182*, 68–79.

Roncarolo, M.G., Levings, M.K., and Traversari, C. (2001b). Differentiation of T regulatory cells by immature dendritic cells. J. Exp. Med. *193*, F5–F9.

Rosenberg, S.A., and Dudley, M.E. (2009). Adoptive cell therapy for the treatment of patients with metastatic melanoma. Curr. Opin. Immunol. *21*, 233–240.

Rosenberg, S.A., Yang, J.C., and Restifo, N.P. (2004). Cancer immunotherapy: Moving beyond current vaccines. Nat. Med. *10*, 909–915.

Sakaguchi, S., Miyara, M., Costantino, C.M., and Hafler, D.A. (2010). FOXP3+ regulatory T cells in the human immune system. Nat. Rev. Immunol. *10*, 490–500.

Sancho, D., Mourão-Sá, D., Joffre, O.P., Schulz, O., Rogers, N.C., Pennington, D.J., Carlyle, J.R., and Reis e Sousa, C. (2008). Tumor therapy in mice via antigen targeting to a novel, DC-restricted C-type lectin. J. Clin. Invest. *118*, 2098–2110.

Sancho, D., Joffre, O.P., Keller, A.M., Rogers, N.C., Martínez, D., Hernanz-Falcón, P., Rosewell, I., and Reis e Sousa, C. (2009). Identification of a dendritic cell receptor that couples sensing of necrosis to immunity. Nature *458*, 899–903.

Schmitt, N., Morita, R., Bourdery, L., Bentebibel, S.E., Zurawski, S.M., Banchereau, J., and Ueno, H. (2009). Human dendritic cells induce the differentiation of interleukin-21-producing T follicular helper-like cells through interleukin-12. Immunity *31*, 158–169.

Schroder, K., and Tschopp, J. (2010). The inflammasomes. Cell *140*, 821–832.

Seder, R.A., Darrah, P.A., and Roederer, M. (2008). T-cell quality in memory and protection: Implications for vaccine design. Nat. Rev. Immunol. *8*, 247–258.

Shaw, J., Wang, Y.H., Ito, T., Arima, K., and Liu, Y.J. (2010). Plasmacytoid dendritic cells regulate B-cell growth and differentiation via CD70. Blood *115*, 3051–3057.

Shedlock, D.J., and Shen, H. (2003). Requirement for CD4 T cell help in generating functional CD8 T cell memory. Science *300*, 337–339.

Shortman, K., and Heath, W.R. (2010). The CD8+ dendritic cell subset. Immunol. Rev. *234*, 18–31.

Siddiqui, K.R., Laffont, S., and Powrie, F. (2010). E-cadherin marks a subset of inflammatory dendritic cells that promote T cell-mediated colitis. Immunity *32*, 557–567.

Siegal, F.P., Kadowaki, N., Shodell, M., Fitzgerald-Bocarsly, P.A., Shah, K., Ho, S., Antonenko, S., and Liu, Y.J. (1999). The nature of the principal type 1 interferon-producing cells in human blood. Science *284*, 1835–1837.

Smyth, M.J., Crowe, N.Y., Hayakawa, Y., Takeda, K., Yagita, H., and Godfrey, D.I. (2002). NKT cells—Conductors of tumor immunity? Curr. Opin. Immunol. *14*, 165–171.

Steinman, R.M., Hawiger, D., and Nussenzweig, M.C. (2003). Tolerogenic dendritic cells. Annu. Rev. Immunol. *21*, 685–711.

Stoitzner, P., Tripp, C.H., Eberhart, A., Price, K.M., Jung, J.Y., Bursch, L., Ronchese, F., and Romani, N. (2006). Langerhans cells cross-present antigen derived from skin. Proc. Natl. Acad. Sci. USA *103*, 7783–7788.

Sun, J.C., and Bevan, M.J. (2003). Defective CD8 T cell memory following acute infection without CD4 T cell help. Science *300*, 339–342.

Takeuchi, O., and Akira, S. (2010). Pattern recognition receptors and inflammation. Cell *140*, 805–820.

Terabe, M., Matsui, S., Noben-Trauth, N., Chen, H., Watson, C., Donaldson, D.D., Carbone, D.P., Paul, W.E., and Berzofsky, J.A. (2000). NKT cell-mediated repression of tumor immunosurveillance by IL-13 and the IL-4R-STAT6 pathway. Nat. Immunol. *1*, 515–520.

Townsend, A.R., Gotch, F.M., and Davey, J. (1985). Cytotoxic T cells recognize fragments of the influenza nucleoprotein. Cell *42*, 457–467.

Trombetta, E.S., and Mellman, I. (2005). Cell biology of antigen processing in vitro and in vivo. Annu. Rev. Immunol. *23*, 975–1028.

Ueno, H., Klechevsky, E., Morita, R., Aspord, C., Cao, T., Matsui, T., Di Pucchio, T., Connolly, J., Fay, J.W., Pascual, V., et al. (2007). Dendritic cell subsets in health and disease. Immunol. Rev. *219*, 118–142.

Ueno, H., Schmitt, N., Klechevsky, E., Pedroza-Gonzalez, A., Matsui, T., Zurawski, G., Oh, S., Fay, J., Pascual, V., Banchereau, J., and Palucka, K. (2010). Harnessing human dendritic cell subsets for medicine. Immunol. Rev. *234*, 199–212.

Valladeau, J., and Saeland, S. (2005). Cutaneous dendritic cells. Semin. Immunol. *17*, 273–283.

van der Aar, A.M., Sylva-Steenland, R.M., Bos, J.D., Kapsenberg, M.L., de Jong, E.C., and Teunissen, M.B. (2007). Loss of TLR2, TLR4, and TLR5 on Langerhans cells abolishes bacterial recognition. J. Immunol. *178*, 1986–1990.

Watanabe, N., Wang, Y.H., Lee, H.K., Ito, T., Wang, Y.H., Cao, W., and Liu, Y.J. (2005). Hassall's corpuscles instruct dendritic cells to induce CD4+CD25+ regulatory T cells in human thymus. Nature *436*, 1181–1185.

Weaver, C.T., Hatton, R.D., Mangan, P.R., and Harrington, L.E. (2007). IL-17 family cytokines and the expanding diversity of effector T cell lineages. Annu. Rev. Immunol. *25*, 821–852.

Welters, M.J., Kenter, G.G., de Vos van Steenwijk, P.J., Löwik, M.J., Berends-van der Meer, D.M., Essahsah, F., Stynenbosch, L.F., Vloon, A.P., Ramwadhdoebe, T.H., Piersma, S.J., et al. (2010). Success or failure of vaccination for HPV16-positive vulvar lesions correlates with kinetics and phenotype of induced T-cell responses. Proc. Natl. Acad. Sci. USA *107*, 11895–11899.

Wykes, M., Pombo, A., Jenkins, C., and MacPherson, G.G. (1998). Dendritic cells interact directly with naive B lymphocytes to transfer antigen and initiate class switching in a primary T-dependent response. J. Immunol. *161*, 1313–1319.

Yamazaki, S., Inaba, K., Tarbell, K.V., and Steinman, R.M. (2006). Dendritic cells expand antigen-specific Foxp3+ CD25+ CD4+ regulatory T cells including suppressors of alloreactivity. Immunol. Rev. *212*, 314–329.

Yewdell, J.W. (2010). Designing CD8+ T cell vaccines: It's not rocket science (yet). Curr. Opin. Immunol. *22*, 402–410.

Zapata-Gonzalez, F., Rueda, F., Petriz, J., Domingo, P., Villarroya, F., de Madariaga, A., and Domingo, J.C. (2007). 9-cis-Retinoic acid (9cRA), a retinoid X receptor (RXR) ligand, exerts immunosuppressive effects on dendritic cells by RXR-dependent activation: Inhibition of peroxisome proliferator-activated receptor gamma blocks some of the 9cRA activities, and precludes them to mature phenotype development. J. Immunol. *178*, 6130–6139.