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Dendritic Cells In Vivo: A Key Target for a New Vaccine Science

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Dendritic cells (DCs) are the antigen presenting cells that initiate and regulate immunity. By studying these cells in vivo, we will be able to move beyond standard approaches and design vaccines that directly harness the elaborate properties of DCs to control immunity.

“The challenge for us as immunologists is to understand how the various elements work and fit together, and then to develop innovative solutions that do better than nature.... Immunity ranks with the most complex of complex systems, along with neurobiology and climate change.” P.C. Doherty and S.J. Turner, *Immunity* 27: 363–365

The traditional vaccines that we know induce immunity against specific microbial antigens and prevent infectious diseases. These vaccines are a major success story and emanate in large part from the discoveries of Louis Pasteur. His first vaccine against chicken cholera was created in 1879, and his most famous vaccine against human rabies was created in 1885 (Dubos, 1988). Pasteur’s research was based on the science of microbiology, i.e., his discovery that distinct microbes are the causes of disease and that an attenuated microbe can induce long-lived protection against infection by the nonattenuated form of that organism. These breakthroughs occurred before there was any clear understanding of vaccine immunity, which began with the discovery of antibodies by von Behring and Kitasato in the 1890s. After the great advances in immunology during the twentieth century, a new vaccine era has finally arrived on the basis of key immune principles.

Vaccines can be defined as formulations that induce specific, nontoxic, and long-lasting immune responses to prevent or treat disease. The new vaccines that immunological research can now develop will deliver the relevant antigens and adjuvants (substances that work with an-

tigens to either enhance or silence immunity) to redirect the immune system for the individual’s benefit (Pulendran and Ahmed, 2006) (Figure 1). The dendritic cell (DC) biology that is described in this issue of *Immunity* provides a foundation on which to create vaccines that not only induce protection against microbes but also deal with cancer (see Melief, 2008), autoimmunity, and allergy (see Belkaid and Oldenhove, 2008).

Here, I will discuss four features of DCs that establish their central role in developing new vaccine strategies: location, antigen handling, maturation, and subsets. As Doherty and Turner write in the introductory quotation, vaccine biology compels us to pull these features of DCs together and “develop innovative solutions that do better than nature.”

Location of Dendritic Cells

In the past, emphasis has been placed on the capacity of DCs to pick up antigens in the periphery, including vaccines at an injection site, and then migrate from peripheral tissues to the T cell areas of lymphoid organs to initiate immunity. The underlying biology is elegant (see Alvarez et al., 2008, this issue), yet more information is needed on the types of DCs that pick up vaccine antigens at an injection site, such as skin or muscle, and initiate immunity. Now additional origins of DCs are apparent (Shortman and Naik, 2007) (see López-Bravo and Ardavin, 2008, this issue). In the steady state, most DCs in lymphoid organs actually arise from a blood precursor (Fogg et al., 2006; Liu et al., 2007). These precursors can proliferate in the lymphoid organ, a process driven by flt-3 ligand (Waskow et al., 2008). Another potentially major source of DCs is

monocytes. Monocyte-derived DCs accumulate in lymphoid tissues during some infections, e.g., *Leishmania major* (Leon et al., 2007). Learning how to mobilize and access these other reservoirs of DCs in vivo could enhance the quality and efficacy of vaccine-induced immunity.

Effective mucosal immunity or resistance at body surfaces is a major hurdle for vaccine development, probably because mucosal surfaces are specialized to sustain nonreactivity to all the innocuous antigens within commensal microorganisms and the proteins in the air we breathe and the foods we eat. HIV-1 vaccines for example will probably need to induce mucosal immunity because this virus is most often transmitted via a mucosal route and quickly leads to a rapid loss of T cells in the intestine (Brenchley et al., 2004; Mehandru et al., 2004). DCs are uniquely located beneath the epithelium at several mucosal surfaces, such as the airway, intestine, and stomach. Intravital microscopy has helped reveal that these cells insinuate their dendritic processes between epithelial cells to enter the mucosal lumen (Chieppa et al., 2006; Niess et al., 2005). A critical goal is to determine whether vaccines can be designed to access these mucosal DCs to bring about better mucosal immunity. Depending upon the medical condition under investigation, desirable mucosal vaccines need to induce both antibodies and T cells that block infection at the site of pathogen entry, or alternatively activate suppressor T cells (“regulatory T cells”), which have the potential to block the inflammatory and allergic diseases at mucosal surfaces (see Belkaid, 2008). One gap in current

knowledge relates to antigen and adjuvant delivery to DCs associated with organized mucosal lymphoid tissues such as Peyer's patches. Because the latter are covered by a distinctive epithelium rich in antigen-transporting M cells, receptors on these M cells, if ligated, could improve vaccine delivery to the DCs that lie underneath (Nochi et al., 2007).

A hallmark of DC location is their abundance in lymphoid tissues, particularly the T cell areas (Alvarez et al., 2008). Numerically, DCs represent a tiny fraction of total cells, but their processes constantly probe the environment, forming a vast and labyrinthine network through which lymphocytes must pass (Lindquist et al., 2004). This sets the stage for the selection of rare but specific clones of lymphocytes during the initial steps of vaccination. It is now feasible to target vaccine antigens directly to these numerous DCs in the T cell areas and modulate their function with adjuvants (Bonifaz et al., 2004; Boscardin et al., 2006; Trumpfheller et al., 2008; Trumpfheller et al., 2006).

Antigen Handling by Dendritic Cells

Because of the molecular identification of the DEC-205 (CD205) antigen uptake receptor on DCs (Jiang et al., 1995), it has become apparent that these cells express a plethora of such receptors, often lectins. These molecules deliver antigens to processing compartments, leading to the presentation of antigen fragments on MHC and CD1 molecules (see Villadangos and Young, 2008). Endocytic receptors in some cases also signal DC activation or deactivation (Robinson et al., 2006). Although DCs are able to capture antigens as solutes in endocytic vacuoles ("pinocytosis"), the identification of uptake receptors changes the study of DC biology in vivo and opens new possibilities for efficient vaccine delivery to DCs and vaccine design.

By identifying specific ligands for antigen uptake receptors on DCs, or by using monoclonal anti-receptor antibodies as surrogate ligands, one can efficiently target vaccine antigens to DCs or their sub-

Some key challenges for vaccines in immunological science and medicine

- What antigens can be protective in microbial and other diseases?
- Can defined adjuvants substitute for complex and replicating microbial vectors?
- How do adjuvants work to facilitate antigen presentation and immune responsiveness?
- What types of lymphocytes provide protection?

Figure 1. Some Key Challenges for Vaccines in Immunological Science and Medicine

sets (see below) in vivo (Hawiger et al., 2001). Retroviral vectors gain improved immunogenicity if the envelope is engineered to target the DC-SIGN (CD209) receptor on DCs (Yang et al., 2008). The efficacy of HIV DNA vaccines can be improved by targeting to DEC-205 (Nchinda et al., 2008). Low doses of protein-based vaccines have also been targeted to DEC-205 along with poly IC as adjuvant. This leads to a large T helper 1 (Th1) cell type of T cell response, whereas targeting to the DCIR2 receptor on another subset of DCs allows both IFN- γ and IL-4 to be induced (Soares et al., 2007; Trumpfheller et al., 2008). Receptor targeting not only enhances antigen uptake and processing a hundred fold but also facilitates research on DCs and receptor function in vivo without the need to isolate the cells (Hawiger et al., 2001)(Bonifaz et al., 2004).

Another major hurdle for vaccine design is "crosspresentation." Nonreplicating vaccines, e.g., the Salk inactivated viral vaccine, as well as protein vaccines, e.g., the diphtheria-pertussis-tetanus (DPT) vaccine, are capable of inducing antibody and CD4⁺ T cell immunity, sufficient in quantity and quality to provide protection against the polio virus and DPT toxins, respectively. However, in order for protein-based vaccines to elicit resistance to HIV and cancer, a nonreplicating form of an antigen needs to elicit MHC-class-I-restricted, CD8⁺ or cytotoxic T cells. Typically, MHC I presentation requires microbial replication and an-

tigen synthesis in an infected cell; e.g., attenuated viral vectors can elicit CD8⁺ cytotoxic T cells, but they also elicit antivector immunity that can compromise the efficacy of booster doses of vaccine. Crosspresentation could overcome these hurdles because it provides a route for "exogenous" vaccine proteins to be processed and to gain access to MHC I. Mechanisms are under study (Kasturi and Pulendran, 2008); the latest concept is that there are special endosomal compartments where internalized antigens are crosspresented (Burgdorf et al., 2008; Di Pucchio

et al., 2008). Certain receptors may be specialized to traverse the crosspresentation pathway, such as Fc receptors for antibody complexes (Dhodapkar et al., 2002a; Regnault et al., 1999), receptors for dying cells (den Haan et al., 2000; Dhodapkar et al., 2002b; Iyoda et al., 2002), and certain C-type lectin receptors such as DEC-205 (Bonifaz et al., 2004; Bozzacco et al., 2007). Targeting these receptors with vaccines may help to overcome the crosspresentation hurdle during vaccination. Nonetheless, I urge the field to move beyond the dominant use of ovalbumin in C57Bl/6 mice as the model antigen for these studies because it is orders of magnitude more sensitive than the antigens that need to be crosspresented to create effective vaccines. Ovalbumin may be distorting the standards for discovering safe, defined, protein-based vaccines.

Maturation of Dendritic Cells

The most intricate feature of DCs is their capacity to differentiate or mature along many different lines. This is driven by many different types of stimuli including (1) microbial ligands for pattern recognition receptors, (2) innate lymphocytes, (3) immune complexes acting on Fc receptors, and (4) additional environmental and endogenous stimuli termed "alarmins." Maturing DCs, depending on the stimulus as well as environmental factors affecting lymphocytes, then determine the type of response, which can be either immunogenic, providing resistance, or

tolerogenic, silencing an immune response. One sphere of immunity that is particularly sensitive to the type of stimulus encountered by DCs is CD4⁺ helper T cell differentiation. The specific pathway followed by CD4⁺ T cells, whether it involves Th1, Th2, Th17, T_H, Tr1, or Treg cell differentiation, is significantly governed by DCs.

A major challenge is to understand how vaccine adjuvants influence DC maturation in vivo, so that the resulting immunity will appropriately resist a particular pathogen or disease. Two important areas of science are pattern recognition receptors and DC subsets (next) (Agrawal et al., 2003; Shah et al., 2003). Synthetic double-stranded RNA, poly IC, is recognized by TLR3 and MDA-5 receptors, and it can polarize CD4⁺ T cells along a Th1 cell pathway when delivered with antigens to the CD205⁺ DC subset (Soares et al., 2007; Trumpfheller et al., 2008). TLR7-TLR8 and TLR9 ligands also can serve as adjuvants for responses by IFN- γ -producing T cells (Wille-Reece et al., 2006). In contrast, ligands for the dectin receptor, when applied to bone-marrow-derived DCs, lead to IL-2 and IL-10 production and seem to favor Th17 cell differentiation (LeibundGut-Landmann et al., 2007). Ligation of c-kit and TSLP receptors allows DCs to induce Th2 cell responses, a process that takes place in the presence of certain allergens such as the house dust-mite allergen (Krishnamoorthy et al., 2008; Soumelis et al., 2002). These observations set the stage to design vaccines that direct the antigen-presenting DCs and/or the responding T cells to develop selected types of immunity; e.g., Th1 T cells help resist many viruses and tumors, Th2 cells mediate parasitic infections, and Th17 cells respond to certain extracellular bacteria and fungi.

An exciting new area will be to design vaccines to specifically silence unwanted immune reactions by taking advantage of DC programs that lead to tolerance. DCs can induce tolerance by deleting or silencing T cells (Brimnes et al., 2003; Hawiger et al., 2004), but they can also induce differentiation of suppressive T cells, e.g., IL-10 producers (Levings et al., 2005; Macdonald et al., 2005), and foxp3⁺ Treg cells (Kretschmer et al., 2005; Luo et al., 2007). Environmental cytokines such as IL-10 and TGF- β , as well as vitamin A, can be critical. The CD8⁺ or CD205⁺ sub-

set of DCs in mice produces more TGF- β (Wang et al., 2008; our unpublished data) and also metabolizes vitamin A to retinoic acid; TGF- β and retinoic acid are cofactors for Treg cell development (Coombes et al., 2007; Sun et al., 2007). A different pathway involves the E-cadherin that DCs use to bind other cells or other DCs; when the DCs detach, the E-cadherin signals upregulation of the lymph-node-homing receptor CCR7 and production of tolerizing amounts of IL-10 (Jiang et al., 2007). In yet another route (and there will be many others!), ligation of select ILT molecules on DCs makes them tolerogenic (Liang et al., 2008; Manavalan et al., 2003). For these reasons, we can start to think about designing vaccines that harness antigen-presenting DCs to regulate and suppress specific, undesirable immune responses in allergy, autoimmunity, and transplantation.

Subsets of Dendritic Cells

Certain markers divide DCs into distinct forms termed subsets (Villadangos and Young, 2008). Many of these markers are receptors involved in pattern recognition and antigen presentation, the key steps in innate and adaptive immunity. All subsets, however, can share characteristic features of the DC lineage such as an unusual, probing dendritic morphology, high amounts of MHC class II, and potent T cell-stimulating activity.

DC subsets are likely to be selected to recognize distinct pathogens or forms of antigen and then follow through with distinct innate and adaptive responses (Liu, 2005; Shortman and Naik, 2007). To illustrate, plasmacytoid DCs express Toll-like receptors (TLR7, TLR8, and TLR9) and respond to viral and self-nucleic acids with vigorous type I interferon production (Kadowaki et al., 2000) (Boonstra et al., 2003) (Boule et al., 2004) (Lande et al., 2007); the plasmacytoid subset also has special endosomal compartments for crosspresentation on MHC I (Di Pucchio et al., 2008). Another subset of CD205⁺ "myeloid" DCs seems specialized to take up and crosspresent antigens from dying cells (den Haan et al., 2000; Iyoda et al., 2002). Within the skin, there are DC subsets that are specialized to induce either cytotoxic T cells or antibody-forming B cells (Klechevsky et al., 2008, this issue). In all these instances, it is likely that the immunogenic DC subset, and conceiv-

ably a tolerogenic DC subset as well, must present the relevant vaccine antigen and directly respond to the vaccine adjuvant (Sporri and Reis e Sousa, 2005; Sporri and Reis e Sousa, 2003). DC subsets therefore greatly expand the number of targets that vaccines can exploit to control immunity.

Monocyte-derived DCs comprise another type of DC subset(s). The term is used in two contexts and their relationship is still not established. In vivo, monocyte-derived DCs were recently described in lymph nodes during infection of mice with the parasite *L. major* (Leon et al., 2007; see López-Bravo and Ardavin, 2008), but their functional properties relative to other DC subsets in lymphoid tissues remain undefined. In addition, for many years scientists have been stimulating monocytes in vitro to develop many features of DCs (Romani et al., 1994; Salustio and Lanzavecchia, 1994). Human monocyte-derived DCs are notably plastic depending upon the type of cytokines in the culture, e.g., IL-4, IL-15, or type I interferon (Dubsky et al., 2007). It still needs to be determined whether a vaccine approach based on monocyte-derived DCs can influence the immune outcome.

DC subsets can vary from one another in all the other features described above: location, receptors, and maturation programs. Nonetheless, given the potential importance of DC-subset diversity in determining the outcome of immunization, particularly the need to generate CD8⁺ cytotoxic T cell immunity in cancer and infections like AIDS, it is unsettling that the information base for DC subsets, including pattern-recognition receptors, is scant for DCs in intact human lymphoid and mucosal tissues.

An Exciting but Challenging Time for Vaccine Science

It is widely acknowledged that fundamental discoveries are needed to develop new vaccines (Klausner et al., 2003). Vaccine science will identify and understand the antigens, adjuvants, and protective cells that provide specific, durable, and non-toxic therapies. In the case of AIDS vaccines, discovery-dedicated academic scientists are finally beginning to receive the support and coordination they require, particularly through the new initiatives of the Bill and Melinda Gates Foundation and the National Institute of Allergy and

Infectious Diseases. Unfortunately, the same cannot be said for other disease targets, particularly cancer. Immunologic approaches to cancer have proven to be effective especially with monoclonal antibodies. However, cellular- and vaccine-based therapies remain underappreciated and poorly supported areas of research, either as stand-alone approaches or in combination with other therapies (see [Melief, 2008](#)). Cancer patients also illustrate most poignantly the difficulties with the currently prevailing therapies against diseases that interact with the immune system. These drugs can have toxic and “off-target” side effects and typically require frequent use. Vaccines, in contrast, offer the ultimate “targeted” therapy because they have the capacity to reach many specific targets in tandem, such as the many alterations in cancer cells, and because they remain directed and durable in their effects.

Vaccine design is often considered an empirical activity. But let us not be derailed by the “empirical” or “descriptive” label. The fundamental challenges are scientific, in that we must discover disease-relevant antigens, adjuvants, and protective mechanisms. Immunology is ready to address these challenges to understand how the various elements work and fit together. This is because the immune system deals directly with the myriad of disease targets, not only hundreds of different infections but also many cancers as well as autoimmune, inflammatory, and allergic conditions.

Yet there are at least three ways in which vaccine science differs from the mainstream of modern immunology. The first is that teams of scientists often have to interact on a regular basis, either within laboratories, between laboratories, or among institutions and countries, to solve the challenges of vaccine biology. There are just too many unknowns when it comes to antigens, adjuvants, and protective immune responses ([Figure 1](#)). Nevertheless, on a more hopeful note as emphasized here, many medical conditions can now be addressed from the focused perspective of vaccination.

The second difference from what immunologists are accustomed to is that vaccine science involves humans, not only mice. I am not referring to large-scale clinical trials testing whether existing practices and concepts are effective.

Rather, we need to work out principles that govern the regulation of the human immune system *in vivo* and integrate this research with other medical sciences such as microbiology, genetics, and cancer biology. Basic research in humans is much more demanding in terms of costs, multiple regulations, and time constraints. Along with our current successful research enterprise, we now need to expand our research to study directly in patients the distinct pathogens and medical conditions that threaten us.

The third difference is that vaccine science involves discoveries that differ from what most scientists are so good at, which is to dissect disease-relevant mechanisms. The other less followed research path, which is imperative for vaccine science, is to identify basic principles that direct immune responses and apply these to “develop innovative solutions that do better than nature.” It is important to dissect immune function in model systems. But it is equally challenging to discover how to direct immunity to create vaccines that guide the human immune system. Dendritic cells, as key orchestrators of these responses *in vivo*, should help in the quest to bring new vaccines into medicine.

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