

## Dendritic Cells and the Control of Immunity:

### Enhancing the Efficiency of Antigen Presentation

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#### Abstract

**Background:** Relevant antigens often are known for diseases that involve the immune system. Yet purified antigens by themselves do not control immunity, especially T-cell immunity. For example, many antigens have been defined for HIV-1 and melanoma, but good HIV-1 vaccines and melanoma immune therapies are lacking. Dendritic cells (DCs) are important intermediaries between antigens and better control of the immune system.

**Methods:** Some properties that allow DCs to control immunity are reviewed, followed by new studies using DCs as adjuvants in humans. An emerging area is then detailed, the special mechanisms whereby DCs enhance the formation of ligands for T-cells, i.e., complexes of major histocompatibility complex (MHC) products and antigenic peptides.

**Results:** Once criteria were developed to identify and isolate DCs, several functional properties became evident. DCs are unusually potent in initiating T-cell mediated immunity in culture. *In vivo*, DCs are positioned to capture antigens and migrate to T-cell areas of lymphoid organs. There, DCs are able to prime animals, controlling the MHC restriction of the primed T-cells and inducing resistance to pathogens. DCs pulsed *ex vivo* with antigens are now being used to induce and expand T-cell immunity in humans. To optimize their use, two areas of DC function need to be harnessed: their terminal differentiation or maturation, and antigen uptake. DCs capture most types of antigens at an immature stage of development, but the cells must receive additional stimuli prior to acquiring potent T-cell stimulatory activity. Stimuli from microbes, inflammation and trauma mature DCs. These change the DCs in several ways, even inducing the formation of MHC II-peptide complexes or T-cell receptor (TCR) ligands. The latter move to the surface in nonlysosomal vesicles that simultaneously carry CD86 costimulatory molecules for T-cell activation. Both MHC and CD86 remain co-clustered in patches at the DC surface. DCs also express a receptor, DEC-205, that enhances antigen uptake and presentation. DEC-205 recycles in an unusual manner through MHC class II-rich, late endosomes or lysosomes, dramatically increasing the presentation of bound ligands. Additionally and importantly, DCs can process dying cells and immune complexes onto MHC class I products, events that are termed the "exogenous pathway" or "cross presentation."

**Conclusions:** The control of the immune system by DCs reflects numerous specializations, not a single "magic bullet." These specializations include a number of mechanisms that increase the efficiency of antigen uptake and MHC-peptide complex formation. The harnessing of these and other features of DCs provides opportunities for improving immune-based therapies and vaccine design.

**Key Words:** Dendritic cells, T-cells, antigen presentation, antigen processing, immune therapy, adsorptive endocytosis, cross presentation, costimulators.

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#### Introduction: Antigens Alone, Even Most Preprocessed Antigens, Do Not Elicit Strong T-cell Immunity

TO INTRODUCE THE FUNCTION OF DENDRITIC CELLS (DCs), consider two situations where dis-

ease and the immune system intertwine. For a tumor like melanoma or a chronic virus infection like HIV-1, there is extensive information on the antigens that T-cells can recognize. However, it is still not feasible to use these antigens to induce strong T-cell mediated immunity in patients and then assess if this increases resistance to tumors and viruses. Likewise in transplantation and autoimmunity, where one wishes to dampen the immune response, antigens are known but the immune system cannot be tolerized. What is the obstacle? The obstacle is that antigens alone, even preprocessed peptides that bind to antigen presenting major

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histocompatibility complex (MHC) class I and II products, are insufficient by themselves to regulate T-cell mediated immunity. This obstacle can be addressed by learning to access and activate DCs, a physiologic system for controlling immunity.

DCs were discovered in an attempt to understand immune priming. Dr. Zanvil Cohn and I were trying to explain a need for non-lymphocytes in a test tube system where sheep red blood cells induced a primary, T-cell dependent, antibody response (1, 2). Dr. Kayo Inaba in Kyoto was also interested in the identical problem (3). Together we found that the required accessories for antibody responses were stellate cells that had not been recognized and characterized before (4, 5). These cells (DCs) were found to have many properties that had not been anticipated: potency in inducing T-cell responses, positioning *in vivo* to capture antigens and migrate to lymphoid tissues, and a capacity to prime CD4 and CD8 types of immunity.

One of the key functions of an accessory cell is antigen uptake and processing to form complexes of MHC products and antigenic peptides. MHC-peptide complexes are the ligands for T-cells. Nevertheless, in much of the early experimentation on DCs, the T-cell stimuli that were used did not require antigen processing, e.g., major transplantation antigens (6, 7), mitogens (8, 9), and contact allergens (10, 11). Therefore, it was assumed that the control of immunity by DCs reflected: (a) their accessory or costimulatory properties, where “costimulation” refers to adhesion and activating molecules that work together with MHC-peptide complexes to activate the T-cell, and (b) *in vivo* functions governing DC position and movement. Recent observations, to be summarized here, now show that DCs are additionally specialized to enhance the efficiency of antigen uptake, processing, and presentation on MHC class I and II products. Other new areas of DC research are not mentioned here. These involve the capacity of DCs to influence Th1/Th2 phase differentiation and tolerance, and other immune cells like B cells, natural killer (NK) and NK-T-cells.

### Some Features Indicating Important Roles for DCs in the Control of Immunity

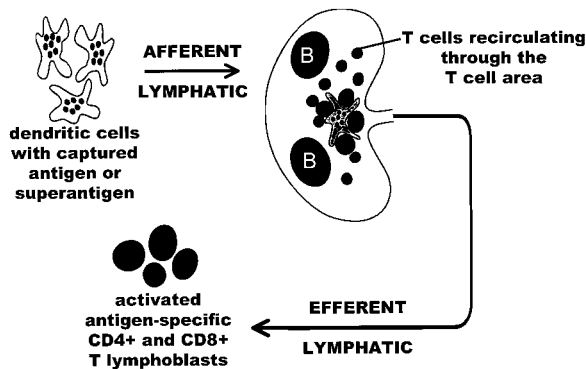
**Potency.** Once DCs were purified, it became evident that they were unusually potent in triggering T-cell clonal expansion and effector function, e.g., CD8+ cytolytic T lymphocytes

and CD4+ helper cells. Small numbers of DCs, and relatively small amounts of antigen, were able to stimulate strong T-cell responses in culture. The basis for this potency is not adequately understood, but two features have recently been reported. One is the selective expression by DCs of a lectin, DC-specific intercellular adhesion molecule 3-grabbing nonintegrin (DC-SIGN), which binds to intracellular adhesion molecule (ICAM)-3 on resting T-cells and enhances the DC-T-cell interaction (12). A second, to be discussed more below, is that the CD86 costimulatory molecule is not only abundant on DCs (13) but also associates with antigen-presenting MHC products in stable patches on the DC surface (14). Therefore, DCs have a specialized T-cell binding molecule, DC-SIGN, and can deliver the key signals for T-cells — MHC and CD86 — in concert.

**Position.** *In vivo* DCs found in the tissues like the skin and airways are positioned to capture antigens. Also DCs can migrate to lymphoid tissues, via lymphatics primarily, to enter the T-cell areas. There, antigen-bearing DCs are ideally located to select antigen-reactive T-cells from the recirculating pool of lymphocytes, after which the immune response can begin (15, 16).

**Priming.** DCs are able to initiate immunity from resting T-cells. The activated T-cells are “MHC-restricted,” i.e., the T-cells recognize antigen in the context of the MHC haplotype of the priming DCs and respond vigorously to antigens presented by these MHC products expressed on other cells (5, 17). In other words, immune responses develop in two stages: an afferent or priming stage that is controlled by DCs, and an efferent stage that eliminates the antigen via other antigen presenting cells like macrophages and B cells. Mature DCs seem to lack many effector or antigen-eliminating functions, such as the capacity to kill microorganisms or make antibodies. Instead DCs activate T-cells, which then turn on other antigen presenting cells, like B cells and macrophages, to eliminate antigens.

Similar events likely transpire *in vivo* (Fig. 1). DCs can be pulsed *ex vivo* with antigens and then reinfused to autologous or syngeneic recipients (18). T-cells are then activated in the T areas. Next, the activated T-cells or T “blasts” leave the lymph node via efferent lymphatics, enter the blood, and finally the inflammatory site where antigen was initially deposited. The use of DCs as adjuvants in mice results in protective immunity against infections (19) and tu-



**Fig. 1.** Two stages of T-cell immunity *in vivo*, the afferent and efferent limbs. In the afferent limb, antigen is picked up by dendritic cells (DCs) that travel to T-cell areas of lymphoid organs, e.g., via afferent lymphatics to the lymph node diagrammed here. In the T-cell areas, DCs select appropriate clones of recirculating T-cells and initiate T-cell expansion and differentiation. Activated T “blasts” leave the lymphoid tissue via efferent lymphatics, enter the blood, and return to the initial inflammatory site to carry out the efferent limb of T-cell immunity together with other antigen presenting cells.

mors (20), but if DCs are carrying an autoantigen, autoimmunity can result (21).

### Using Dendritic Cells as Adjuvants in Humans

Given the information in the previous section, it is now feasible to start thinking about the issues posed at the beginning of this article. Can one use DCs to induce stronger immunity to virus infections and tumors in humans? This is a vigorous research area (22, 23) and not simply a matter of “translation” of existing knowledge from mouse to man. More often, key findings in DC biology are being made with humans before being “translated” back into the mouse.

A key obstacle to human DC research has been overcome, at least in part. This is the preparation of large numbers of DCs for purposes of vaccination. Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a critical cytokine, being used to expand DCs from proliferating progenitors (24, 25), and for differentiating DCs from nonproliferating monocyte precursors (26, 27). The first successful immunization studies with monocyte-derived DCs, loaded *ex vivo* with antigens, expand CD4+ and CD8+ T-cell immunity upon reinfusion *in vivo* (28, 29). Likewise CD34-derived DCs have been used to expand immunity to melanoma antigens in patients with advanced cancer (30).

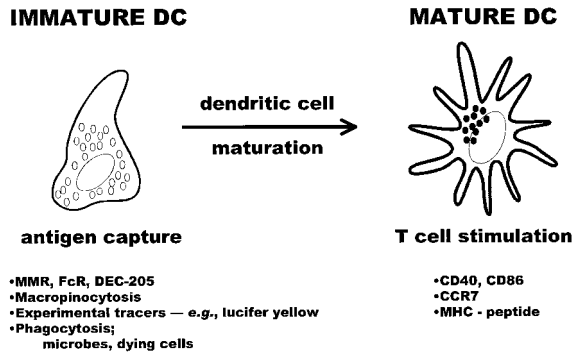
There are many ways in which this DC-based, active-immunization approach can be

improved. It may be necessary to select for certain types of DCs or specific stages of development. More mature DCs are more immunogenic in mice (31) and in humans (32). It may be important to prolong the life span of the DC (e.g., by treatment with CD40L or TRANCE), since normally most DCs in an injected inoculum do not survive more than 2 days (33). Methods for loading DCs with antigens also need research. It would seem important to load the DCs with multiple epitopes, and on several types of antigen presenting molecules (including MHC class I and class II products), to induce helper and killer cells for many tumor epitopes. Finally, one needs to be able to measure immunity to be able to optimize the use of any adjuvant. The measurement of the human immune response is becoming increasingly quantitative, e.g., through the use of fluorescent-activated cell sorting (FACS) approaches that measure MHC-tetramer-binding and cytokine-producing T-cells.

### Specializations of DCs in Converting Antigens into Immunogens

**The Concept of DC Maturation.** The terminal stage of DC differentiation, prior to death by apoptosis, is a cell that expresses very high levels of antigen presenting MHC products as well as the accessory or costimulatory molecules for T-cell binding and activation. These mature cells arise from immature precursors which, importantly, are the cells that exhibit endocytic activity (Fig. 2). Endocytosis occurs via several pathways: fluid phase pinocytosis including macropinocytosis (34); receptor-mediated uptake via Fc receptors and lectins like the macrophage mannose receptor (MMR) (34); and phagocytosis of microbes and dying cells (35–37) (Fig. 2). In other words, many populations of DCs separate in time their two main functions: antigen capture as immature cells and immune stimulation as mature DCs.

Maturation is, therefore, a critical control point in the initiation immunity. It can be mediated by inflammatory cytokines (interleukin-1 [IL-1], tumor necrosis factor [TNF]), T-cell products (CD40L), microbial constituents (lipopolysaccharide [LPS], CpG oligonucleotides), and trauma (necrosis, transplantation). Maturation leads to the development of many features critical for priming, not just costimulators. For example, maturing DCs produce IL-12, resist immunosuppression by IL-10, and express different chemokine receptors to



**Fig. 2.** Dendritic cell maturation, a key control point linking innate and adaptive immunity. Immature DCs are active in endocytosis. There are several adsorptive endocytosis receptors like the MMR, FcR and DEC-205, and DCs that can take up large amounts of substrates (including experimental tracers) by macropinocytosis and phagocytosis. Many stimuli can drive differentiation to form mature or T-cell stimulatory DCs. The stimuli include microbial products (LPS and dsRNA); CpG deoxyoligonucleotides; TNF family members including CD40L on activated T-cells, platelets, mast-cells; and trauma (necrosis and transplantation). Maturing DCs produce large amounts of IL-12 and, in some cases, type I interferons and become specialized in many ways to stimulate T-cells. For example, mature DCs have high levels of T-cell interaction molecules like CD40 and CD86, an important chemokine receptor CCR7, and high levels of MHC-peptide complexes or TCR ligands.

orchestrate their movements and interactions with lymphocytes *in vivo*.

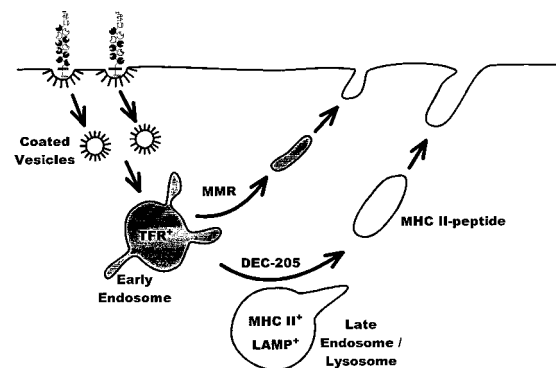
In the past 2 years, it has become apparent that immature DCs are not just “transient phagocytes.” Instead they have some unusual ways to enhance antigen uptake and formation of immunogenic MHC-peptide complexes or T-cell receptor (TCR) ligands. Several of these mechanisms are now considered.

**The DEC-205 Multilectin Receptor.** Monocyte-derived DCs can express two homologous adsorptive endocytosis receptors, the MMR and DEC-205, with 8 and 10 contiguous C-type lectin domains respectively. Natural ligands for the MMR and DEC-205 on DCs are at this time insufficiently characterized. Nonetheless, in spite of sequence similarities whereby both molecules display many contiguous C-type lectin domains, the MMR and DEC-205 can be quite different biologically. In particular, the receptors are found in different intracellular sites within developing mouse DCs (38). As expected, the MMR is abundant within peripheral early endosomes, through which most adsorptive endocytosis receptors recycle. In contrast, DEC-205 is abundant in late endosomes or lysosomes, which in DCs are rich in antigen presenting MHC class II products. These MHC II compartments (MIICs) are

the sites in which peptides, formed during lysosomal proteolysis, bind to MHC II molecules prior to exit to the cell surface (14, 31).

To understand the contrasting intracellular distribution of MMR and DEC-205, Karsten Mahnke engineered the cytosolic domains of the MMR and DEC-205 together with the external domains of human CD16. Following transfection into L cells, he could then study the trafficking that is mediated by each tail, and mutants thereof, in the absence or presence of human Ig as ligand (38). Mahnke et al. found that the MMR “tail” trafficked CD16 through early endosomes, whereas the DEC-205 cytosolic domain trafficked CD16 through late endosomes or lysosomes identified with the marker, LAMP, or lysosome-associated-membrane protein (Fig. 3). In both cases, the MMR and DEC-205 tails returned the CD16 to the cell surface to pick up more ligand, human Ig. In the DCs and L cells that we studied, the compartments targeted by DEC-205 also were rich in MIIC products. This feature of an endocytic receptor, i.e., recycling through late endosomes and lysosomes (Fig. 3), has not been seen before.

When aggregated human Ig or Ig-anti Ig immune complexes were next tested as antigens for T-cells primed to human Ig, the L cells that expressed CD16-DEC-205 presented antigen 100 times more efficiently than L cells expressing CD16-MMR. An acidic EDE sequence in the distal part of the DEC-205 tail was critical,



**Fig. 3.** Distinct intracellular trafficking mediated by the cytosolic domains or “tails” of the MMR (macrophage mannose receptor) and DEC-205 adsorptive endocytosis receptors. The MMR tail, when hooked to the external domains of a human CD16 Fc receptor, recycles CD16 into and out of the cell through early endosomes. This pathway is typical for adsorptive endocytosis receptors, as first noted with low-density lipoprotein receptors. DEC-205, in contrast, traffics via late endosomes or lysosomes, marked by LAMP (lysosome-associated-membrane proteins), that importantly, are rich in MHC class II. This greatly enhances the efficiency of antigen presentation via the DEC-205 cytosolic domain. TFR<sup>+</sup> = transferrin receptor.

both for targeting CD16 to MHC II and LAMP-positive compartments and for enhancing antigen presentation.

**The Exogenous Pathway for Presentation on MHC Class I.** In most antigen-presenting cells, a tiny fraction of endocytosed substrates can be processed into peptides that bind to MHC class II but not to MHC class I. For MHC class I-peptide complexes to form, the peptides are derived from newly synthesized or “endogenous” proteins in the cytoplasm, which are processed via the proteasome and transported into the endoplasmic reticulum (ER) via transporters for antigen processing (TAP). However, the laboratories of Nina Bhardwaj (36, 39) and Sebastian Amigorena (40, 41) have shown that DCs do efficiently present “exogenous” substrates from dying cells and immune complexes onto MHC class I (Fig. 4). Presentation has been shown to be TAP-dependent in the case of immune complexes. Somehow, DCs are able to deliver endocytosed proteins, not just *de novo* synthesized proteins, into the MHC I pathway. The pathway is also called “cross presentation” (cross priming and cross tolerance) because antigens from dying cells of one MHC haplotype can be presented by different MHC molecules expressed by DCs.

Importantly, cross presentation of dying cells is also very efficient for MHC II products on DCs (37, 42). We studied the capacity of mouse DCs to present a peptide derived from I-E MHC II products in dying B cells. If we fed recipient DCs dying B cells, the donor I-E peptide gained access to the MHC II of the DCs more than 1000-fold more efficiently than preprocessed I-E peptide. In other words, when DCs are feeding on dying cells, they should efficiently generate MHC II-restricted CD4 helper cells, whereas the administration of preprocessed peptide will be much less effective. Since the peptide we studied derived from

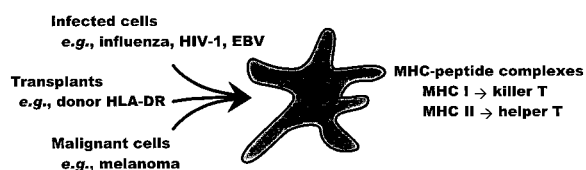
MHC class II itself (37), our observations should bear upon the “indirect” pathway of transplant rejection. In this pathway, donor-derived MHC products are presented by the recipient, most likely recipient DCs, setting up a means for chronic rejection.

Several examples are being found in which human DCs process antigens from tumor cell lines. DCs process essential latency antigens (EBNA1, LMP1, LMP2, EBNA3) from Epstein Barr virus-transformed, B-lymphocyte cell lines onto MHC class II (42, 43). Likewise, DCs process antigenic peptides from melanoma cell lines, for presentation on MHC class I and priming of the T-cell response, even with T-cells from patients with advanced melanoma (44). Clinical studies will eventually determine if the delivery of dead autologous or allogeneic tumor cells to DCs, followed by reinfusion, provides a means to immunize a patient to an array of tumor antigens on both MHC class I and class II.

In summary, the capacity of DCs to process dying cells is deservedly drawing enormous attention. It may lie at the heart of many challenging problems in medicine: the indirect pathway of transplant rejection, self-tolerance initiated via presentation of dying self-tissues, immunity to viral and bacteria-infected cells that undergo cell death, and tumor immunity (Fig. 4).

**Regulating Antigen Presentation during DC Maturation.** Protein entry into the endocytic system is typically followed by presentation of at least some processed peptides on MHC II. However, a fascinating form of regulation of antigen processing and presentation has been found when DCs internalize the protein Hen Egg Lysozyme (HEL) into their lysosomal MIICs. These MHC II-rich compartments are called lysosomes (late endosome might prove to be a more accurate term for these vesicles) because they stain brightly for lysosome-associated membrane proteins or LAMPs and for the peptide-editing MHC product, H-2M or HLA-DM.

Using the C4H3 monoclonal antibody specific for an epitope formed by the complex of HEL peptide and MHC II, we looked for sites of MHC-peptide or TCR-ligand formation in DCs from mouse bone marrow (31). We found that immature DCs endocytosed the HEL into MIICs, as expected, but the cells had to receive a maturation stimulus to form large amounts of C4H3 epitope, i.e., MHC II-peptide complex. This was a nice demonstration that complexes are first found within deep endocytic compartments as suspected, but surprisingly, the forma-



**Fig. 4.** Dendritic cell presentation of dead and dying cells from various sources to form MHC class I and class II-peptide complexes. This pathway is likely to be very important clinically, because it allows DCs to process antigens from cells that are dying within infectious foci, transplants and tumors. DCs are dying quantitatively and perhaps qualitatively distinct in their capacity to form TCR ligands by the exogenous pathway.

tion of MHC-peptide complexes was not simply a matter of delivering antigen to MIICs. Instead, the formation of TCR ligands was maturation stimulus-dependent and could take place if the stimulus was delivered days after entry into the MIIC. Therefore, the endocytic system of immature DCs is not an obligatory site for protein digestion and TCR ligand formation; both seem to be regulated in some way.

Dr. Ira Mellman and his colleagues have found that one level of regulation is very likely at the level of proteolytic activity. The MIICs of immature DCs contain abundant cystatin C, a cysteine protease inhibitor that blocks the function of cathepsin S (45). The latter is critical for invariant chain proteolysis, allowing antigenic peptides to exchange in the MHC II peptide-binding groove with the class II-associated invariant chain peptide (CLIP) fragment of the invariant chain. Accordingly, preprocessed peptides should load the MHC II products of DCs well when a maturation stimulus is also administered; this was indeed observed by Inaba et al. (31).

Maturing DCs also exhibit a beautiful mechanism for the transport of MHC-peptide complexes to the cell surface (14). Following their formation in MIICs, the MHC-peptide complexes are found in abundance in peripheral, nonlysosomal compartments. These vacuoles do not accept most endocytic tracers and rather seem to be transport vesicles, bringing TCR ligands from late endosomes to the cell surface and leaving behind residual endocytosed proteins, HLA-DM, and LAMPs. The term immunogenic "TCR ligand" is really appropriate for the content of these vesicles, because both MHC I and II are abundant, as are the costimulator molecule B7-2 or CD86. Subsequently, at the cell surface the DCs display large amounts of MHC and CD86 ("signals one and two" for T-cell activation) together. This would also allow the DC to set up the immunologic synapse in responding T-cells. The idea of the synapse is that the TCR must cluster to signal well, and that in addition costimulators like CD86 must be juxtaposed to function optimally. If so, DCs that have picked up antigens in the presence of a maturation stimulus are able to display the processed antigens and other T-cell activation signals in a manner that is prone to synapse formation.

### Discussion

Successful manipulation of the immune system in the context of many diseases will require,

I think, successful manipulation of DCs. Well-defined antigens, coupled with current artificial adjuvants, exhibit poor efficacy. Logically, one should optimize the form of antigen, so that it targets to DCs and efficiently charges antigen-presenting molecules like MHC I and II. Lectin receptors, Fc receptors, and receptors for dying cells all could be valuable in this regard. Then one must mobilize and mature the DC, but do this in concert with antigen uptake. In this way, many elegant features come together at the level of the DC. These include the successful processing of antigens and display of MHC-peptide complexes, and the expression of many other ligands and receptors that orchestrate interactions with T-cells, especially *in vivo*.

We have concentrated on immunogenicity, the clonal expansion and priming of effector T-cells, taking the lead from the melanoma and HIV-1 examples mentioned initially. However, the function of DCs extends beyond this traditional emphasis. DCs can stimulate other types of lymphocytes (e.g., B cells, NK cells, NK-T-cells), and different forms of innate and adaptive (Th1/Th2, tolerance) immunity. Therefore, by learning to manipulate DCs *in vivo*, it should be possible to control many components of the immune response. Additional research on DCs will allow nature's adjuvants to carry out the physiology for which they are beautifully designed.

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