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## Some interfaces of dendritic cell biology

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RALPH M. STEINMAN

Laboratory of Cellular Physiology and Immunology and the Chris Browne Center for Immunology and Immune Disease, The Rockefeller University, New York, USA

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The field of dendritic cell (DC) biology is robust, with several new approaches to analyze their role *in vivo* and many newly recognized functions in the control of immunity and tolerance. There also is no shortage of mysteries and challenges. To introduce this volume, I would like to summarize four interfaces of DC research with other lines of investigation and highlight some current issues. One interface is with hematopoiesis. DCs constitute a distinct lineage of white blood cell development with some unique features, such as their origin from both lymphoid and myeloid progenitors, the existence of several distinct subsets, and an important final stage of differentiation termed “maturation,” which occurs in response to inflammation and infection, and is pivotal for determining the subsequent immune response. A second interface is with lymphocyte biology. DCs are now known to influence many different classes of lymphocytes (B, NK, NKT) and many types of T cell responses (Th1/Th2, regulatory T cells, peripheral T cell deletion), not just the initial priming or induction of T cell-mediated immunity, which was the first function to be uncovered. DCs are sentinels, controlling many of the afferent or inductive limbs of immune function, alerting the immune system and controlling its early decisions. A third interface is with cell biology. This is a critical discipline to understand at the subcellular and molecular levels the distinct capacities of DCs to handle antigens, to move about the body in a directed way, to bind and activate lymphocytes, and to exert many quality controls on the type of responses, for both tolerance and immunity. A fourth interface is with medicine. Here DCs are providing new approaches to disease pathogenesis and therapy. This interface is perhaps the most demanding, because it requires research with humans. Human research currently is being slowed by the need to deal with many challenges in the design of such studies, and the need to excite, attract and support the young scientists who are essential to move human investigation forward. Nonetheless, DCs are providing new opportunities to study patients and the many clinical conditions that involve the immune system.

Key words: DC subsets; DC maturation; peripheral tolerance; endocytosis; immune therapy; vaccine; exogenous pathway.

Ralph M. Steinman, The Rockefeller University, New York, NY 10021-6399, USA.  
e-mail: steinma@mail.rockefeller.edu

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### DCs AND HEMATOPOIESIS: THE INTRICATE DEVELOPMENT AND LIFE HISTORY OF DCs

Initially, DCs were noted to be bone marrow derived and to share a progenitor with macrophages and granulocytes in colony forming assays. Granulocyte-macrophage colony stimulating factor (GM-CSF) was recognized as a key stimulating cytokine. The DCs were regarded to be a separate cell lineage, because their prop-

erties were so distinct from phagocytes, in particular the potency with which DCs stimulated T cells and their capacity to initiate immune responses in culture and in mice. This initial view of DC development, i.e., as a parallel track to the development of macrophages and granulocytes, has proven to be oversimplified. The life history of DCs is replete with distinctive and sometimes perplexing features, so much so that this section of the introduction will be the longest.

*Early progenitors.* To begin, DCs can originate from either common lymphocyte or common myeloid progenitors (1). These progenitors have been isolated from the bone marrow using a panel of markers. Both progenitors share a lack of the "lineage" markers of differentiated hematopoietic cells, but express IL-7 receptor in the case of lymphoid progenitors and c-kit in the case of myeloid progenitors. The terms "lymphoid" and "myeloid" are best reserved to denote DCs that derive from these distinct precursors. However, the consequences of this dual origin of DCs are not yet evident. In fact, it is still difficult to identify the progeny of lymphoid and myeloid DC developmental pathways in intact tissues, and functional distinctions remain to be established.

*Subsets.* Another unusual feature of DCs is that there are several subsets, as assessed by surface markers and functions (reviewed in (2)). The existence of subsets was first emphasized in mice, where two major groups of CD8<sup>+</sup> CD4<sup>-</sup> and CD8<sup>-</sup> CD4<sup>-</sup> DCs were distinguished (3). Subsets of DCs have also been identified in the rat, marked as CD4<sup>-</sup> OX41<sup>-</sup> and CD4<sup>-</sup> OX41<sup>+</sup> (4, 5). Perhaps these rat subsets correspond to the CD8<sup>+</sup> and CD8<sup>-</sup> mouse DC subsets (CD8 is not found on rat or human DCs). A major functional difference between these subsets is that the mouse CD8<sup>+</sup> and rat CD4<sup>-</sup> DCs selectively take up certain types of dying cells (6–9).

In both mice and humans, there are additional DCs called "plasmacytoid" which are distinguished from other DCs, often termed "myeloid". This term leaves something to be desired, because it implies that plasmacytoid DCs are nonmyeloid. This is not established, and a myeloid origin is actually suggested by the expression in plasmacytoid DCs of c-fms, the receptor for macrophage colony stimulating factor (10). I suspect the term "plasmacytoid DC" will remain useful, since it portrays their resemblance to plasma cells, which led to their original definition; other DC subsets may need to be denoted with more specific terms such as "CD8<sup>-</sup> mouse DCs".

Importantly, DC subsets are now proving to have major "innate" differences involving cytokine production, receptors for antigen uptake, and receptors for microbial ligands (Toll-like receptors), cytokines and chemokines. The De-

cember 2002 volume of Human Immunology has an entire issue devoted to plasmacytoid DCs. These cells, for example, are able to make unusually high amounts of interferon- $\alpha$  in response to viral infection (11, 12), to express a distinct marker called BDCA-2 that is capable of endocytosis and antigen presentation (13) (although note should be taken that plasmacytoid DCs are very weak at capturing soluble and particulate antigens), to respond to IL-3 rather than GM-CSF (14), and to express Toll-like receptor 9 that responds to bacterial DNA and demethylated CpG deoxyoligonucleotides (15, 16). Other DCs can make large amounts of IL-12 in response to bacterial cell wall components (17–19), express a number of endocytic receptors that are not found on plasmacytoid DCs such as Langerin and an asialoglycoprotein receptor (20–22), respond to GM-CSF rather than IL-3 (23), and express Toll-like receptors 2, 3 and 4 (24).

There are additional DC subsets that have recently been identified. One is found in human blood and is marked by a 6-sulfo LacNAc carbohydrate modification of the selectin ligand PSGL-1; these DCs are capable of producing large amounts of TNF (25). In the cornea, there are abundant immature DCs that lack detectable MHC class II molecules; however, following transplantation, these DCs undergo typical differentiation to express characteristically high levels of MHC class II (26). There also are CD11c<sup>+</sup> DCs that develop during infection and move from blood into infected lymphoid and nonlymphoid tissues (27–29).

It remains unclear whether DC subsets have distinct patterns of migration in vivo. Some of the current findings are as follows. Plasmacytoid DCs have a long life span relative to other subsets of DCs (30, 31). Perhaps this long life span is associated with a continuous recirculation from blood through lymphoid organs via high endothelial venules, for which plasmacytoid DCs express the CD62L selectin homing molecule. Many DCs can express CLA-4 (25). This is an epitope on PSGL-1, a ligand for CD62E and CD62P, i.e., the E and P selectins expressed by vessels in peripheral tissues. Some DCs rapidly extravasate into tissues in the steady state, but this occurs by a PSGL-1-independent mechanism (32, 33). Plasmacytoid DCs also can be found in an extravascular location in con-

ditions such as nasal allergy and cutaneous lesions of lupus erythematosus (34, 35).

Another subset distinction originates from work on DCs within the skin, but it may apply to DCs from other organs. Epidermal DCs (Langerhans cells) express high levels of markers, such as CD1a, Langerin and E-cadherin; these are not found on most DCs in the dermis and interstitial spaces of other organs (dermal or interstitial DCs). Reciprocally, dermal DCs can express DC-SIGN/CD209, the macrophage mannose receptor/CD206, and CD13. One interesting functional distinction between epidermal and dermal DCs is that the latter stimulate B cells in certain assay systems (36).

It was originally thought that Langerhans cells are immediately derived from monocytes. However, their numbers in the steady state are maintained by local proliferation in the skin; only during inflammation is there recruitment of Langerhans cells from CCR2-bearing precursors from the blood and bone marrow (37), possibly from DCs expressing CD1a (38). TGF $\beta$  has a major effect on LC development (39–43), and this cytokine may additionally induce the CCR6 chemokine (for CCL 19 or MIP-3 $\alpha$ ) receptor (44) for homing to a variety of epithelia.

Much of the information on DC subsets at this time is derived from cells isolated *ex vivo*. It will be valuable to learn to manipulate these subsets *in vivo*, to determine their consequences for tolerance and immunity. One way that may help in this regard is to learn to selectively deliver an antigen to a DC subset, as can now be done with dying cells that selectively target the CD8<sup>+</sup> subset of mouse DCs, for example (8, 9). The plethora of DC subsets identified with current markers can seem confusing, but the data are suggesting that the different subsets at a minimum are specialized for recognizing distinct pathogens and carrying out distinct innate functions. As learned from the history of lymphocyte subsets, the initial markers that define a subset eventually allow for experiments that yield clearer criteria applicable to intact animals and patients.

*Expansion.* DC numbers *in vivo* can be greatly expanded with flt-3L and also with G-CSF (45–48). Flt-3L is especially important in expanding

the numbers of plasmacytoid DCs, including from bone marrow precursors *in vitro* (23). When these cytokines are used systemically, including in humans, the expanded cells are mainly in their precursor and immature stages of development. Additional strategies will likely be necessary to translate these impressive increases in DC numbers into improved immune control *in vivo* (49).

*Blood monocyte precursors.* Blood monocytes can give rise to macrophages, but they also serve as an intermediary in DC development. The bipotential nature of monocytes first became evident *in vitro*, using GM-CSF plus IL-4 (or IL-13) to induce DC differentiation (50, 51). There seem to be subsets of monocytes, given new evidence that cells expressing the CD16 Fc $\gamma$ R seem precommitted to differentiate into DCs (52). In contrast, different inflammatory and infectious stimuli may drive most monocytes to develop into either macrophages or DCs. For example, LPS and certain LPS-bearing bacteria block the conversion of phagocytic monocytes to DCs (53). Therefore, on the one hand blood monocytes include subsets precommitted to become either macrophages or DCs in the steady state, while, on the other hand, most monocytes under specific conditions may develop either into DCs (*ex vivo* GM-CSF and IL-4) or macrophages (intracutaneous LPS-bearing bacteria).

*Migration.* The migration and positioning of DCs are among the hallmarks of this lineage. Different chemokine receptors are valuable at different stages of the life history of DCs. For example, CCR2 seems important for DCs to translocate into the T cell rich regions of lymphoid tissues during contact allergy and infection with *L. major* (54); CCR5 may help recruit DCs to inflammatory sites (55, 56); CCR6 appears to be important for positioning DCs at epithelial surfaces (57–59); CCR7 may increase entry to lymphatics and migration to the T cell areas of lymph nodes (60). Other mediators influence DC migration perhaps by controlling the function of chemokine receptors, such as lipids like lipoxins, leukotrienes, prostaglandins (61, 62).

There may be some misunderstanding that DCs only migrate into lymphatics during inflammation and infection. However, DCs seem

continuously on patrol through peripheral organs, lymph and lymphoid tissues in the steady state. This steady state migration provides DCs an opportunity to sample self antigens and environmental proteins continuously for purposes of immune tolerance (6, 63). There is new evidence that the uptake of apoptotic cells can increase expression of CCR7 (a chemokine receptor that mediates homing to the T cell area), without driving other features of DC maturation (below) (64). Also, the accumulation of CCR7<sup>+</sup> but otherwise immature DCs occurs in certain clinical states associated with increased numbers of Langerhans cells in lymph nodes (65). One possibility is that DCs (or a subset of DCs) entering the lymph in the steady state are cells that have taken up dying cells and started to express CCR7.

*Transcriptional controls.* The analysis of DC development requires more data on transcriptional controls. Several are under study, with the NF- $\kappa$ B/rel family providing intriguing guidelines already. There are several NF- $\kappa$ B proteins, which in turn can form a number of heterodimers. These different rel family members contribute to different stages of DC development. Mice lacking rel B lack the CD8<sup>-</sup> subset of spleen DCs (66), while mice lacking both rel A (p65) and p50 lack most DCs, probably because of diminished survival (67). Mice lacking cRel and p50 fail to respond to TRANCE and CD40L to control survival and cytokine (IL-12 p40) production by maturing DCs (67).

Interestingly, DCs express high levels of all NF- $\kappa$ B proteins, not just activities (68). This may help to explain how DCs react so quickly and vigorously to many stimuli that signal through NF- $\kappa$ B, for example Toll-like and TNF-family receptors.

DCs are nonproliferating cells that are quickly responsive to different environmental stimuli. Because some types of DCs are available in large numbers e.g., monocyte-derived DCs, they may be ideal to study the regulation and recruitment of stimulus-dependent transcriptional factors to chromatin, e.g., the p38 MAP kinase-dependent recruitment of NF- $\kappa$ B (69). The new methodologies for studying access and binding of transcriptional factors to their promoters promise to accelerate this field of research and to identify distinct signal transduction pathways

that control sets of genes during DC differentiation.

*Life span.* The function of DCs, especially mature DCs (see below), is significantly influenced by their life span and survival, which can be extended through TNF-family receptors like TRANCE-R (RANK) and CD40 (70, 71). The need to gain better control of DC migration and survival arises during immunotherapy, where DCs charged with antigens *ex vivo* are being used to elicit immunity, especially tumor-specific immunity (below).

*Maturation.* A major feature of the life history of DCs is termed maturation. The events that take place before and during DC maturation are important in understanding the control of immunity and tolerance. When *ex vivo*-derived, antigen-pulsed DCs are used to immunize mice (72, 73) or humans (74, 75), the mature DCs are immunogenic, whereas immature DCs can induce regulatory cells (76). Formal proof that DC maturation *in vivo* (rather than *ex vivo*) controls the induction of CD4<sup>+</sup> and CD8<sup>+</sup> T cell immunity was only recently obtained. The experiments used  $\alpha$ -galactosylceramide, which acts via NKT cells to mature DCs (77). These DCs, following antigen capture and maturation *in vivo*, were able to induce immunity upon adoptive transfer to naive animals, without an additional antigen or maturation stimulus.

Immature DCs are adept at capturing antigens, especially through receptor-mediated uptake (below). Also, immature DCs respond quickly and vigorously to many microbial and inflammatory (type I and II interferons, GM-CSF, TNF $\alpha$ ) stimuli via Toll-like and cytokine receptors. Nevertheless, the single term "immature" is used to describe DCs in different circumstances, e.g., the Langerhans cells of the epidermis, the cells generated from monocytes with GM-CSF and IL-4, and many of the DCs within lymphoid organs in the steady state. These DC populations likely have differences, e.g., in their capacities to form MHC-peptide complexes (see below). However, each of these immature DCs captures antigens and responds rapidly to maturation stimuli to become potent stimulators of immunity.

During maturation, DCs dampen endocytic receptor expression and endocytosis itself (78),

and they undergo wholesale changes in the expression of many molecules used to interact with T cells, such as several B7-family members (CD80, CD86, PD-L2/B7-DC, ICOS-L), TNF family members (CD137/4-1BBL, CD134/OX40L, CD70), as well as chemokine receptors (CCR5, CCR7). The term maturation initially was meant to highlight the intricate and multifaceted nature of DC differentiation (79–83). Now it is clear that dozens of genes are altered in their expression in maturing DCs at least *ex vivo* (84–86). This exciting area now needs to be addressed more fully *in vivo*.

There are many stimuli for DC maturation. Many microbial ligands and synthetic compounds act on distinct Toll-like receptors to control DC maturation, e.g., viral RNA and poly IC on TLR3 (87), mycobacterial extracts on TLR2 and TLR4 (88), imidazoquinolines on TLR7 (18, 89, 90), and bacterial DNA and CpG deoxyoligonucleotides on TLR 9 (91, 92). Physiologic molecules like  $\beta$ -defensins may also exploit Toll-like receptors in order to mature DCs (93). Other maturation stimuli signal DCs through pathways distinct from the Toll-like receptors. These include Fc $\gamma$  receptors for immune complexes (94, 95), and PIR-B (96) and TREM-2 (97) whose ligands are not yet known; CD100 (98); and several TNF family members like TNF $\alpha$  itself, fasL (99) and CD40L. CD40 signaling in particular is a powerful inducer of most of the immunogenic functions of DCs (100–102) and also stimulates DC maturation and immunogenicity *in vivo* (8, 103, 104). Innate cell types like NK cells (105, 106),  $\gamma\delta$  T cells (107) and NKT cells (77, 108) can mature DCs by pathways that remain to be worked out. An important finding is that certain types of necrotic cells can induce maturation *in vitro* (109). This may require the function of inflammatory cytokines like TNF (110) or heat shock proteins (111), the latter acting at the level of CD40 (112) and possibly through Toll-like receptors (113, 114). Type I interferons, which can be produced in abundance by plasmacytoid DCs (11, 12), and to some extent by other DCs (25), are emerging as significant inducers of DC maturation (115–118). IFN $\alpha$  induces DC maturation in two longstanding settings of immune enhancement, i.e., the spontaneous maturation of certain types of DCs in culture (119), and the adjuvant action of complete Freund's adjuvant

(120). Actually, the above lengthy summary of inducers of DC maturation does not represent a complete summary of the literature.

It seems unlikely that functionally identical DCs will develop in response to the breadth of distinct maturation stimuli just outlined above. Nonetheless, each stimulus can provide control of the immune response through the aegis of DC maturation. It may be important to stress that the critical cell biology linking innate and adaptive immunity is the process of DC maturation, rather than signaling via Toll-like or other specific receptors *per se*. Signaling other cell types via these receptors does not generate cells with the potent and specialized properties of DCs, which additionally are able to control the quality of the adaptive response, as we shall discuss below.

In a reciprocal sense, many agents are being defined that block DC maturation. Most notable are a number of pathogens, which may evade the immune system by blocking maturation (reviewed in (121)), as well as some natural compounds such as  $1\alpha$ , 25-dihydroxyvitamin D3 (122, 123). There also are mechanisms to dampen select components of DC maturation, particularly IL-12 production (124–127). Papers have begun to appear on the pharmacologic blockade of DC maturation (122, 128, 129), a strategy that may reduce chronic inflammatory disease and the initiation of transplant rejection.

There has been a major change in emphasis with the new information that immature DCs are not simply ignored but instead are able to mediate tolerance in the steady state, e.g., by a deletional mechanism (8, 103, 104). The term “mature” in a functional sense has always connoted immunogenicity, e.g., the differentiation and expansion of effector functions and memory. This concept of maturation was one of the first to distinguish antigen handling by immature DCs, which is required for antigen-specific tolerance, from the many other accessory functions used by DCs and other cells to control immune responsiveness (82, 83). Admittedly, the two terms – immature and mature – are insufficient to precisely describe the different kinds of DCs that have been generated *in vitro* or have been isolated from different tissues *in vivo*. There is interest in identifying new terms to describe different states of DC function, but I

would like to suggest that rather than more descriptive nomenclature, it might be more fruitful to concentrate on defining specific stimulatory pathways and molecular markers for different functional states of DCs.

#### DCs AND THE QUALITY OF THE IMMUNE RESPONSE: MORE THAN POTENT PRIMING OF T CELLS

The initial hallmarks of DC function involved the induction of T cell responses, especially the potency of DCs as stimulators of T-dependent antibody production (130) and T cell proliferation (131, 132), and the capacity to prime or initiate immunity (133, 134). Now it is evident that DCs coordinate many additional adaptive functions.

*Effects on different classes of lymphocytes.* DCs can contribute to the expansion and differentiation of most classes of lymphocytes, not just T cells, but also B cells (135–138) and innate NK (105, 106, 139, 140) and NKT cells (77, 108, 141). Rather little is known about the mechanisms for stimulating these other lymphocytes, although the TNF family members BAFF/Blys and APRIL (137, 142) as well as cytokines (143) are implicated in B cell stimulation.

Another important sphere is to understand DC numbers and function at mucosal surfaces, within or adjacent to the lining epithelia as well as the mucosal-associated lymphoid tissues (144–146). Mucosal DCs, because of tissue-related environmental stimuli (such as in the lung or intestine), may be in a “regulatory” mode in the steady state. The mucosal DCs are able to induce regulatory T cells that prevent immune reactivity to harmless environmental antigens (147), or alternatively, these DCs may be differentiated to polarize T cells towards the Th2 helper pathway (148).

*The induction of CD4<sup>+</sup> Th1 and Th2 cells.* DCs can control the Th1/Th2 quality of the T cell response (reviewed in (149)). The efficiency with which DCs can induce Th1 type immunity has been shown in humans (74), even in patients with advanced cancer (75). I am not aware of another adjuvant that leads so quickly to the Th1 pathway of CD4<sup>+</sup> T cell differentiation.

There is a mystery here, however. Th1 differentiation can be driven by IL-12 produced by DCs (150–152), whereas the fully mature DCs that have induced Th1 responses in human are thought to be “exhausted” in terms of their capacity to produce IL-12 (153). Possibly exhausted DCs retain sufficient IL-12-producing capacity to differentiate T cells along the Th1 pathway. Alternatively, other pathways or cytokines, such as IL-23 (154), may mediate Th1 development by DCs.

Th2 responses also can be fundamentally dependent upon DCs. Several pathogens, e.g., schistosomes (155, 156), filaria (157), fungi (158, 159) and cholera toxin (160), utilize DCs to induce classical Th2 responses. Two new mediators have been identified that may act on DCs in the setting of allergy to induce Th2 responses. One is histamine, which when added to monocyte-derived DCs in the presence of lipopolysaccharide reduces IL-12 production and the induction of Th1 responses, while enhancing IL-10 production and Th2 development (161). A second mediator is human thymic stromal lymphopoietin, which is of epithelial origin and is a DC-based inducer of “inflammatory” Th2 responses (162). This cytokine selectively binds to a subset of DCs, which then acquire the capacity to induce Th2 cells that produce IL-4. However, in contrast to “standard” Th2 cells, these T cells fail to produce IL-10 and instead make high levels of TNF, hence the appellation “inflammatory” Th2 cells. Another intriguing feature of Th2 development relates to the lung. Antigens administered via the nasal route often induce Th2 type responses, and this seems to require special functions of DCs in the lung environment (148).

Thus DCs and DC subsets are adaptive, able to respond quickly and in many ways to the environment. A good deal of the current literature on Th1/Th2 differentiation utilizes DCs that are handled *ex vivo*, rather than DCs that are studied directly *in vivo*. Nevertheless, the quality of the T cell response likely is influenced by several factors that act at the level of DCs, such as antigen dose, microbial stimuli that induce Th1-polarizing cytokines (IL-12, IL-23, IFN- $\alpha$ ), and cytokines made by surrounding cells (163). This “sensor” function of DCs illustrates their sentinel role in immunity.

*T cell memory.* A critical element in the quality

control of an immune response is the establishment of memory. New roles for DCs are becoming evident. DCs have the capacity to produce IL-15 (164–166) and thereby sustain memory CD8<sup>+</sup> T cells (167), but IL-15 production by DCs has not been pursued sufficiently *in vivo*. Nonetheless, DCs can select for higher affinity memory cells in both humans and mice (168–171). Additionally, when DCs have captured antigens in the lung, they have the capacity to persist there for up to a month (172). Thus, there are hints that DCs can play several direct roles in establishing memory.

It is of further interest that CD4<sup>+</sup> T cells seem to be required to sustain strong CD8<sup>+</sup> T cell memory under several circumstances, e.g., resistance to persistent herpes viruses (173). Possibly the CD4<sup>+</sup> cells are able to sustain DC function, e.g., at the level of IL-15 production, and in turn the memory CD8<sup>+</sup> T cells. One feature of DC function in this regard is their capacity to present antigens on both MHC class I and II products, including the exogenous pathway in which DCs capture dying cells during infection, tumors and transplants. Conceivably, the improved targeting of vaccines and maturation stimuli to DCs (below) will initiate combined CD4<sup>+</sup> and CD8<sup>+</sup> T cell responses and improved memory against chronic infections and tumors.

*Peripheral T cell tolerance.* DCs can influence peripheral tolerance, and by more than one mechanism. *In vivo*, there is evidence that immature DCs control deletional tolerance in the steady state, in the absence of inflammation or infection (8, 103, 104). The DCs in lymph nodes endocytose and process antigens to form MHC-peptide complexes in the steady state. T cells then respond to these complexes by proliferation but then are deleted. In contrast, immunity develops under conditions where the DCs are exposed to maturation stimuli, such as triggering of CD40.

In addition to deletional pathways, DCs can induce regulatory and suppressor T cells. In tissue culture, immature monocyte-derived DCs can induce IL-10-producing regulatory CD4<sup>+</sup> T cells (174), while plasmacytoid DCs have been shown to induce IL-10-producing CD8<sup>+</sup> T cells (175). In mice, DCs from the lung are able to expand CD4<sup>+</sup> regulatory T cells, and this re-

quires expression of both IL-10 and ICOS-L in the DCs (147). IL-10-producing DCs also induce regulatory CD4<sup>+</sup> T cells that reduce inflammation in candidiasis (176). In humans, an injection of peptide-pulsed immature DCs leads to the formation of antigen-specific regulatory CD8<sup>+</sup> T cells (76). New evidence indicates that DCs are able to expand antigen-specific CD4<sup>+</sup> CD25<sup>+</sup> suppressor T cells (177). More work is needed on the capacity of DCs to generate different types of regulatory T cells *in vivo*. These may originate from distinct CD25<sup>+</sup> and CD25<sup>+</sup> precursors (178, 179), but both have the potential to dampen immune responses in critical settings such as autoimmunity, tumors, transplantation, and chronic inflammation and infection (reviewed in (180)).

Experiments with DCs may help to resolve some longstanding challenges in peripheral tolerance. First, is it possible for low doses of intact antigens to silence the immune system? Experimentally, high doses of soluble proteins and, more often, preprocessed peptides are used to induce tolerance, but it seems important that high affinity T cells remain tolerant to small amounts of self and environmental proteins. Recently, DCs have been shown to mediate peripheral tolerance when targeted with low doses of cell-associated antigens, or with soluble antigens captured by the DEC-205 receptor (8, 103, 104). Second, current treatments in transplantation and inflammation globally block lymphocyte co-stimulation and cytokine production. It would be exciting to design antigen-specific strategies for immune suppression. DC-based tolerance, either deletional or regulatory, offers the potential to manipulate the immune response in an antigen-specific manner. Third, there are many pathways to immune or antigen-specific tolerance. It is important to understand how to control these pathways *in vivo*, as now seems feasible with DCs in the case of the peripheral deletion of naïve T cells (8, 103, 104) and possibly regulatory T cells (76, 147). Fourth, DCs create a danger when they mature in response to an infection, because they are capturing a mixture of microbial, self and environmental antigens. Is it possible that DCs help to solve their own dilemma by tolerizing T cells to harmless antigens prior to infection, so that the T cell repertoire is either deleted or silenced to the harmless antigens by the time that

infection takes place? All in all, the control of tolerance is like the control of immunity. In addition to antigens and lymphocytes, it is important to include DCs in the analysis.

### CELL BIOLOGY AND DCs: THEIR DISTINCTIVE "PARTIAL" FUNCTIONS

Paul Ehrlich in his Nobel lecture in 1908 said, "even now the time has come to find a way into the finest chemistry of cell life, and to dissect the inclusive concept of the cell into a large number of single and specific partial functions." This is the essence of modern cell biology, which began almost 40 years later. DCs are proving to have many exciting "partial functions", some of which are emphasized here. These need to be pursued to identify mechanisms underlying much of the physiology outlined above.

*Antigen capture.* To begin, DCs are specialized for antigen capture, particularly at the immature stage of development. Antigens can be taken up as solutes during fluid phase pinocytosis and macropinocytosis, as ligands for specific endocytic receptors, and as particles. The specialized antigen uptake capacities of DCs represent a relatively new perspective, since historically DCs were uncovered as potent stimulators of responses to stimuli that did not require processing, such as T cell responses to allogeneic cells (131), superantigens (181), and mitogens (82, 132). These mature cells were ostensibly weak in endocytic activity. The distribution and migration of DCs in situ then became evident, showing that DCs could migrate via the lymphatics to the T cell areas of lymph nodes to initiate immunity. Given these special features as "nature's adjuvants", very little emphasis was initially given to the possibility that DCs would have additional specializations for antigen capture and processing, as now appears to be the case.

A critical feature in evaluating antigen capture is to utilize immature DCs. An increasingly studied example involves dying cells (7, 8, 182–189), although requisite uptake receptors in vivo have been difficult to pinpoint (190, 191). DCs also have receptors for heat shock proteins, which can deliver peptides for presentation on MHC class I products (192, 193), with the scav-

enger receptor LOX-1 being an important candidate in the case of Hsp70 (194). Many of the molecules used to identify DCs have cytosolic domains with endocytic motifs, e.g., DEC-205 (195, 196), Langerin (20), asialoglycoprotein receptor (22), and DC-SIGN (197, 198). It will be demanding but valuable to identify natural ligands for these receptors. Then it will be feasible to better target antigens to DCs. Also, these receptors may help to understand how different complex antigens, especially infectious agents, interact with DCs as illustrated by DC-SIGN below.

In the absence of information on natural ligands, antibodies to DC receptors are proving useful as surrogate ligands to initiate the study of function in vivo. Introduction of antigens into an antibody to the DEC-205 endocytosis receptor enhances the efficiency of antigen presentation by DCs to CD4<sup>+</sup> and CD8<sup>+</sup> T cells at least several hundred fold (103, 104). In the steady state, antigen targeting to DCs leads to tolerance; DC maturation seems essential to induce effector cells and memory. The combination of antigen targeting to DC plus a DC maturation allows cell-associated antigens to induce protective anti-tumor immunity in mice (77).

*Antigen processing.* Once antigens are taken up, DCs seem to process them efficiently for presentation on MHC class I, II and CD1 molecules. The successful processing of antigens to MHC-peptide complexes or TCR ligands can be observed directly in DCs (72, 199). Nevertheless, I feel there is a major dichotomy between DCs derived from cultured marrow precursors and DCs in the lymphoid tissues. Antigen processing in marrow and monocyte-derived DCs is exquisitely regulated by maturation stimuli such as lipopolysaccharide and TNF family members. The regulation is evident at the level of MHC peptide complex formation, which in turn reflects regulation of such elements as the protease inhibitor cystatin C (200), the proton pump in lysosomes (201), and the expression of TAPs (202, 203). In contrast, DCs within T cell areas are able to process antigens into functional MHC-peptide complexes in the steady state, in the ostensible absence of inflammation and infection. This is indicated by the comparable stimulation of T cell proliferation when

antigen is presented in the steady state or in the presence of a maturation stimulus (8, 103, 104).

For MHC class I, DCs should help to work out the still mysterious "exogenous pathway" for processing many different types of nonreplicating ligands, such as immune complexes, dying cells, inactivated microbes and vaccines, and DEC-205 ligands. The exogenous pathway can be totally dependent upon TAP transporters of antigenic peptides (8, 104), although a TAP-independent pathway also has been described (204). The processing of exogenous nonreplicating antigens seems valuable in many instances of tolerance and immunity, including antigens delivered as immune complexes (95, 205–207), dying infected cells (182, 184, 186), allogeneic cells (7, 183, 189), tumors (185, 187, 207) and self tissues (8, 188, 208, 209).

It is important that DCs can process complex antigens, such as infectious agents and dying cells, to peptides that are presented on both MHC class I and II products. An intriguing recent example of the potential in vivo relevance of the exogenous pathway is the capacity of DCs to prime human CD4 and CD8 T cells to antigens in the Epstein Barr virus (210). T cell-mediated immunity prevents the development of virus induced lymphoproliferation and malignancy, but it has been found that T cells are not primed directly by EBV-infected B cells, even though the latter are often termed "professional antigen presenting cells". Instead the processing of dying B cells by DCs is required to prime CD4<sup>+</sup> and CD8<sup>+</sup> virus-specific T cells, and both classes of T cells serve to resist B cell transformation with EBV in vitro. In general, the exogenous pathway to MHC class I seems to be best developed in DCs, and DCs can be essential for this pathway in vivo according to recent results (211).

*Binding and immunologic synapse formation with T cells.* The remarkable capacity of DCs to initiate the clustering and activation of T cells still needs to be unraveled in mechanistic terms. A longstanding idea is that DCs first bind to T cells in an antigen-independent manner, and that this allows the two cells to scan each other for a "match" between presented MHC-peptide complexes and the TCR (212–215). There are some recent insights on possible molecular mechanisms for such "scanning" or "antigen-

independent clustering." One mechanism involves DC-SIGN/CD209 on the DC interacting with ICAM-3/CD50 on the resting T cell (216, 217). Another is the neuronal molecule, neuropilin-1, now implicated in the initial DC-T cell interaction (218). Neuropilins typically interact with semaphorins, but in the case of DC-T cell interactions, a homophilic interaction between neuropilin on the DC and T cell has been suggested. More information is needed on the role of DC-SIGN and neuropilin in vivo.

The TCR engagement that follows the antigen-independent DC-T interaction is likely to be accompanied by formation of an immunologic synapse, a large tight contact zone. Synapse formation is often studied with B cells as presenting cells, but more recently DCs have been considered (219). Also, many of the experiments with B cells have utilized pre-activated T cells as responders, in which the properties of the TCR and other adhesion molecules may have changed from the naive or unprimed T cell. In contrast, DCs may be specialized to form synapses with naive T cells, for example, following successful antigen processing, DCs export clusters of MHC peptide and CD86 to the cell surface, which then may set up the supra-molecular activation complex on T cells (199, 220). Synapse formation with DCs may soon be approached in vivo given the new methods to observe DC function in intact living lymphoid organs (221, 222).

*Costimulatory B7 and TNF family members.* There are many mysteries with respect to the B7 family, many members of which can be expressed at high levels on DCs. Initially, the B7 family and its functions were quite simple. There were two members, CD80 and CD86 (originally referred to as B7-1 and B7-2). These were expressed at high levels on DCs, particularly after maturation, and they functioned to costimulate T cell activation (223, 224), especially the high level production of IL-2 that typifies T cell stimulation by DCs (225, 226). The situation is proving to be much more intricate. To illustrate, CD80 and CD86 are expressed at significant levels by immature DCs in lymphoid tissues. T cells proliferate vigorously to antigens presented by these immature DCs but are then tolerized; strong IL-2 production and immunity only develop when a maturation

stimulus is given, and the levels of CD80 and CD86 increase a further 5–10 fold (8, 103, 104). The function of CD80 and CD86 on DCs in vivo remains to be studied directly. Then there are new B7 family members that are quite distinct. B7-DC (also referred to as PD-L2) is a B7 family member that is upregulated markedly by some stimuli for DC maturation (227). This molecule may have both inhibitory (228, 229) and activating (230) roles in the immune response. The former likely involves B7-DC signaling via the inhibitory PD-1 counter-receptor on the T cell, while activation might involve a distinct receptor on the T cell or perhaps a response within the DC (230). ICOS-L is another B7 family member. When expressed by DCs in the lung, it can play a role in the induction of regulatory rather than effector T cells (147). For all these B7 family members, there needs to be much more in vivo work to understand their contribution to DC function in different immunologic circumstances.

For TNF family members, 4-1BBL and OX40L are both expressed on maturing DCs. 4-1BBL could be a critical player in the long term expansion and persistence of CD8<sup>+</sup> memory T cells (231, 232), while OX40L may stimulate helper T cells to migrate to B cell follicles during a T-dependent antibody response (233). BAFF, Blys, and APRIL are TNF-like molecules that allow DCs to stimulate T-independent antibody responses (28, 137). Other TNF family members also need to be considered from a DC perspective, such as GITR-ligand (234, 235) whose functions in vivo are under investigation.

All in all, there are many distinct B7 and TNF family members expressed by DCs. Their regulation will be important to pursue, since they could be vital to understanding the quality controls of the immune response that are exerted by DCs.

*Secretory products of DCs.* An intriguing secretory product of DCs (as well as other cells) is the exosome, small 50–90 nm vesicles that carry MHC and costimulatory molecules (236). Exosomes made by one population of DCs can be presented to T cells by other DCs, the latter acting as a “platform” to display MHC-peptides (and very likely other molecules) from the exosomes (237). Exosomes that are prepared ex

vivo and injected into mice also are presented to T cells. The extent to which this fascinating form of antigen transfer influences immune physiology in vivo is not yet apparent, but studies are already underway to test exosomes as a “freeze dried form of DCs” with which to stimulate cancer-specific T cells in patients (238).

DCs secrete a number of other products, beginning with several chemokines and cytokines. Their rate of secretion varies with the maturation state of the DCs. Many chemokines are produced in high amounts during a short (hours) period following receipt of a maturation stimulus (153). Likewise for IL-12, high levels are produced early after the onset of maturation, especially upon receipt of a combination of a microbial and CD40 stimulus (239) (perhaps to induce the expression of both p40 and p35 subunits). In spite of these kinetics, it may not be accurate to conclude that mature DCs are exhausted with respect to their capacity to elicit Th1 type CD4<sup>+</sup> T cell responses, something at which they seem to excel in vivo in humans (74, 75). The control of IL-10 production by DCs (240) is important to decipher, given the potential of IL-10 to help elicit antigen-specific regulatory T cells (147). IL-2 also can be produced early during the maturation of some DCs, and this contributes to T cell (84) and NKT cell (141) responses. In summary, DCs can produce several different cytokines and thereby exert important quality controls on the immune response.

Of new interest are the noncytokine growth and suppressive factors made by DCs. Growth factors include thiols (241), while suppressive factors include the products of indoleamine 2,3-dioxygenase (242–244), an enzyme that is expressed in DCs (245) and is upregulated during ligation of certain B7 family members on DCs (243). Therefore, DCs employ several secretory and cell surface molecules to control the quantity and quality of the immune response.

*Molecules shared with the nervous system.* To conclude this sampling of cell biological issues, an interesting new partial function of DCs is likely to become apparent as a result of their expression of such molecules as neuropilin-1 (218) and semaphorins A and D (CD100) (98, 246), identified initially for their controlling role

in axon guidance. Their role on DCs *in vivo* remains to be studied. Years ago, the term “dendritic” may have been a confusing choice to identify a new hematopoietic lineage, because of extensive prior use of the term in neuroanatomy. Given these new interfaces between the immune and nervous systems, “dendritic” may prove to have more breadth than originally intended.

#### DCs AND DISEASES THAT INVOLVE THE IMMUNE SYSTEM: APPROACHES TO PATHOGENESIS AND PROTECTIVE MECHANISMS

DC biology provides opportunities to study some of the most challenging areas of medicine. Reciprocally, the many clinical problems that involve the immune system keep us mindful of the amount and type of knowledge that is needed.

*Atopic disease and allergy.* There are exciting data on a DC pathway to inflammatory allergic diseases, such as asthma and atopic dermatitis (162). In this pathway, thymic stromal lymphopoietin is produced by epithelial cells and acts on IL-7-like receptors expressed by a subset of DCs in blood. This allows the DCs to become stimulators of a distinct type of Th2 cell in which both IL-4 and TNF, but not IL-10, are expressed.

*Lupus erythematosus.* Another area of pathogenesis involves autoimmunity. It has been shown that monocytes from patients with lupus acquire some of the features of DCs (247), primarily the result of excess IFN- $\alpha$  found in this systemic autoimmune disease (248). In mice, such chronic DC activation leads to intense autoimmunity, including features of lupus (249). From a therapeutic perspective, there is another side of DC function in autoimmunity, i.e., the potential to use DCs to control different types of tolerance (above), both deletional and suppressive.

*Vaccines for infectious diseases.* With respect to enhancing immunity in the setting of vaccines against infectious diseases and their toxins, some important possibilities are emerging to better exploit DCs as nature’s adjuvants (re-

viewed in (250)). There are examples *in vivo* for enhancing DC numbers, stimulating DC maturation, and targeting antigens to DCs and DC subsets. Importantly, DCs have the potential to process complex but nonreplicating antigens into peptides that are presented on several types of antigen presenting molecules, not only MHC class II products but also MHC class I and CD1 molecules. In mice, DNA vaccines may gain their efficacy by being able to transduce DCs in small numbers (251–253) and in addition to mature the cells (254) through TLR9 receptors. However, these experiments in mice need to be pulled together into bone fide vaccine strategies, especially in humans.

*Microbial pathogenesis.* Although DCs valuably sense the presence of pathogens and initiate innate and adaptive protective responses (reviewed in (121)), these cells also can be exploited by infectious agents at three levels. First, several pathogens – from viruses to plasmodia – are able to interfere with DC maturation and presumably thereby evade immunity. Second, pathogens can utilize DCs for replication, transport and transmission. The most notable route currently is via DC-SIGN, a C-type lectin expressed on some DC subsets. DC-SIGN already has been implicated in infections caused by HIV-1 (198, 255–257), Ebola virus (258), Dengue virus (259), cytomegalovirus (260), Mycobacterium tuberculosis (261, 262), and a species of Leishmania amastigote (263). Other infectious agents can be carried via DCs, e.g., prions (264), but the mechanism and consequences of this transport are not yet evident. Third, it has been proposed that DCs have the potential to induce peripheral tolerance to infectious agents (265). This remains to be established directly as a mechanism for DC-based, microbial pathogenesis *in vivo*.

*Cancer immunotherapy.* DCs are being used to actively immunize patients against their cancers. In general, the DCs are generated *ex vivo* and charged with tumor antigens prior to reinfusion. The studies are proceeding slowly because of the demands of doing research with patients. There is much to learn with these *ex vivo* approaches, e.g., to study different subsets and maturation states of DCs, and to better control DC migration and survival as mentioned above

(reviewed in (266, 267)). There have been interesting phase I and II studies in which a significant number of patients with advanced cancer have been immunized to tumor antigens delivered by autologous DCs, some of whom have undergone clear tumor regression (268–276). Phase III DC therapy trials can now be designed. Whatever the initial results will be, the field has considerable potential for further investigation of the immune response to human cancers, because immunotherapy will now be directly investigating the natural adjuvants of the immune system. There are also clues on how to coax DCs to take up tumor cells and to mature in situ (277), i.e., to try to exploit DCs more effectively against tumors in vivo without having to use the ex vivo approach of loading DCs with antigens and then reinfusing the cells.

Again, the current approaches to DC therapy are likely to be suboptimal, since the field is in its early stages, there are many variables to work out, and the experiments require research with patients. Also, cancers will most likely possess evasion mechanisms to evade the responses induced by DCs, although it should be kept in mind that DCs have the potential to elicit a number of different resistance mechanisms involving T, B, NK and NKT cells. I feel we should take a positive perspective to the study of DCs as adjuvants for generating cancer-directed immunity. I say this because it is now feasible to use DCs to analyze and manipulate specific responses to human tumor antigens in patients, as opposed to injecting tumor cells or tumor antigens without deliberately trying to mobilize the many adjuvant roles of DCs.

For example, patients with advanced melanoma can be induced through DCs to expand melanoma-specific immunity in vivo (75, 271). T cells from patients with progressive multiple myeloma (including T cells from the bone marrow environment that is infiltrated with malignant plasma cells) can develop strong cytolytic T cell responses to authentic tumor cells, when their T cells are primed ex vivo with DCs loaded with antibody-coated myeloma (278). I think that the myeloma findings provide the first example that T cells from a tumor bed, and not just a rare T cell line, can be reliably stimulated en masse to kill freshly isolated tumor targets, at least in culture. Ex vivo-based DC therapy makes it possible to control DC maturation and

deliver a broad spectrum of tumor antigens to DCs, as with RNA and whole tumor cells. Both of these new potentials could lead to improve outcomes with immunotherapy and immunoprevention. These different strategies illustrate the theme that tumor immunology, like immunity and tolerance to other antigens, should benefit by experiments that directly address the DC sentinels in addition to the standard emphases on tumor antigens and lymphocytes.

## DISCUSSION

The articles that follow provide in depth reviews on specific areas of DC function and, as a group, illustrate some valuable features of DC research: the study of complex antigens such as infectious agents and tumor cells; the search for new ideas relating to pathogenesis and therapy of disease; and the in vivo analysis of innate and adaptive immunity. The need for more in vivo information has been repeatedly stressed here.

Several approaches are likely to be valuable such as gene arrays to monitor transcriptional activity (84, 86), gene targeting through promoters that are active in DCs (211, 279, 280), two-photon microscopy of living lymphoid tissues (221, 222), and manipulation of human immune response with ex vivo-derived and in vivo-targeted DCs (reviewed in (266, 267, 281)).

This introduction tries to portray some of the challenges in DC biology by looking at its interfaces with other disciplines. I think it is rewarding for DC research to be able to interface with such other fields as hematopoiesis, cell biology and lymphocyte biology, but also to set the bar at a high level to obtain sufficient knowledge to influence disease.

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