

# IMMUNOBIOLOGY OF DENDRITIC CELLS

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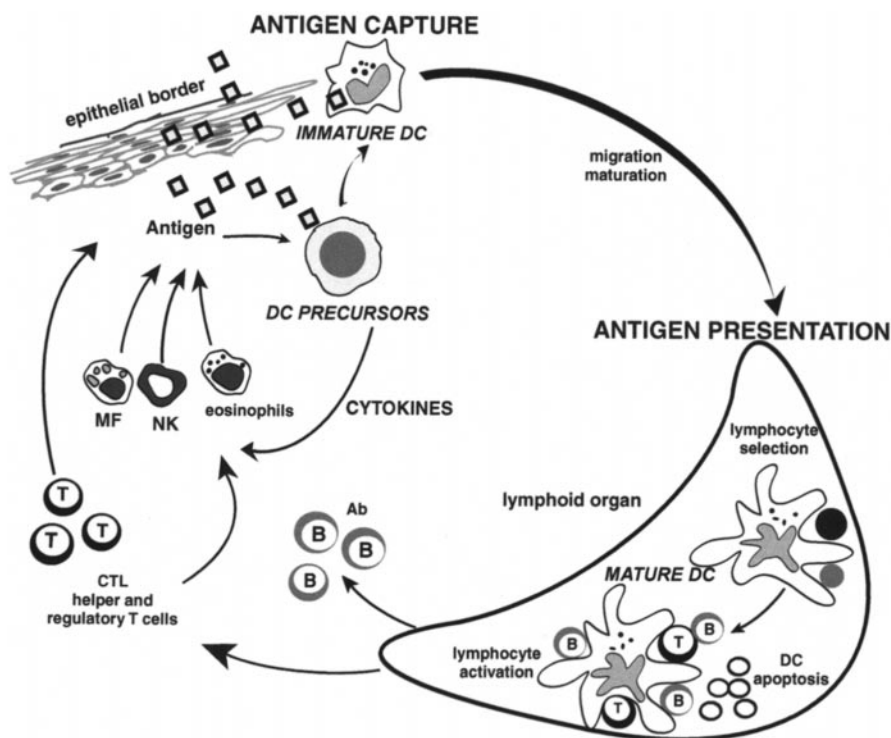
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■ **Abstract** Dendritic cells (DCs) are antigen-presenting cells with a unique ability to induce primary immune responses. DCs capture and transfer information from the outside world to the cells of the adaptive immune system. DCs are not only critical for the induction of primary immune responses, but may also be important for the induction of immunological tolerance, as well as for the regulation of the type of T cell-mediated immune response. Although our understanding of DC biology is still in its infancy, we are now beginning to use DC-based immunotherapy protocols to elicit immunity against cancer and infectious diseases.

Host defense relies on a concerted action of both antigen (Ag)-nonspecific innate immunity and Ag-specific adaptive immunity (1–3). Key features of the mammalian innate immune system include (a) the ability to rapidly recognize pathogen and/or tissue injury and (b) the ability to signal the presence of danger to cells of the adaptive immune system (4). The innate system includes phagocytic cells, natural killer (NK) cells, complement, and interferons (IFNs). Cells of the innate system use a variety of pattern recognition receptors to recognize patterns shared between pathogens, for instance bacterial lipopolysaccharide (LPS), carbohydrates, and double-stranded viral RNA (5–7). Evolutionary pressure has led to development of adaptive immunity, the key features of which are (a) the ability to rearrange genes of the immunoglobulin family, permitting creation of a large diversity of Ag-specific clones and (b) immunological memory. Yet this highly sophisticated and potent system needs to be instructed and regulated by Ag-presenting cells (APCs). Dendritic cells (DCs) are unique APCs because they are the only ones that are able to induce primary immune responses, thus permitting establishment of immunological memory (8–11). DC progenitors in the bone marrow give rise to circulating precursors that home to tissues, where they reside as immature cells with high phagocytic capacity (Figure 1). Fol-

lowing tissue damage, immature DCs capture Ag and subsequently migrate to the lymphoid organs, where they select rare Ag-specific T cells, thereby initiating immune responses. DCs present Ag to CD4<sup>+</sup> T-helper cells, which in turn regulate the immune effectors, including Ag-specific CD8<sup>+</sup> cytotoxic T cells and B cells, as well as non-Ag-specific macrophages, eosinophils (12), and NK cells. Moreover, DCs educate effector cells to home to the site of tissue injury. Four stages of development have



**Figure 1** The life cycle of dendritic cells (DC). Circulating precursor DCs enter tissues as immature DCs. They can also directly encounter pathogens (e.g. viruses) that induce secretion of cytokines (e.g. IFN $\alpha$ ), which in turn can activate eosinophils, macrophages (MF), and natural killer (NK) cells. After antigen capture, immature DCs migrate to lymphoid organs where, after maturation, they display peptide-major histocompatibility complexes, which allow selection of rare circulating antigen-specific lymphocytes. These activated T cells help DCs in terminal maturation, which allows lymphocyte expansion and differentiation. Activated T lymphocytes migrate and can reach the injured tissue, because they can traverse inflamed epithelia. Helper T cells secrete cytokines, which permit activation of macrophages, NK cells, and eosinophils. Cytotoxic T cells eventually lyse the infected cells. B cells become activated after contact with T cells and DCs and then migrate into various areas where they mature into plasma cells, which produce antibodies that neutralize the initial pathogen. It is believed that, after interaction with lymphocytes, DCs die by apoptosis.

been delineated, including (a) bone marrow progenitors; (b) precursor DCs that are patrolling through blood and lymphatics as well as lymphoid tissues, and that, upon pathogen recognition, release large amounts of cytokines, e.g. IFN- $\alpha$ , thereby limiting the spread of infection; (c) tissue-residing immature DCs, which possess high endocytic and phagocytic capacity permitting Ag capture; and (d) mature DCs, present within secondary lymphoid organs, that express high levels of costimulatory molecules permitting Ag presentation (Figure 1). DCs constitute a complex system of cells which, under different microenvironmental conditions, can induce such contrasting states as immunity and tolerance. This review summarizes recent progress in our understanding of DC development and immunoregulatory functions. For earlier references regarding DC biology, the reader is invited to consult the most recent reviews (8–11).

## HETEROGENEITY OF DENDRITIC CELL SUBSETS

### Mice

At least two distinct pathways of DC development have been identified in mice, myeloid and lymphoid. Evidence for the myeloid origin of DCs comes mainly from *in vitro* studies in which myeloid-committed precursors give rise to both granulocytes/monocytes and myeloid DCs under the influence of granulocyte/macrophage colony-stimulating factor (GM-CSF) (13, 14). DCs can also arise from lymphoid-committed precursors (15–19). Transfer of a population of purified lymphoid precursors into irradiated hosts results in the development of T cells, B cells, NK cells, and DCs that express CD8 $\alpha$ , but not cells of the myeloid lineage (15, 19). The ability to generate DC but not NK or B cells is maintained by the downstream CD44<sup>+</sup>CD25<sup>+</sup>CD3<sup>−</sup> pre-T cell population, suggesting that DCs are more closely linked to T cells than to NK or B cells (19, 20). However, a strict clonal analysis showing that DCs arise from the same precursor cells as NK cells or T cells has not yet been done. Although the lymphoid origin of DCs has been demonstrated only for CD8 $\alpha$ <sup>+</sup>, the similarity in phenotype of thymic DCs to CD8 $\alpha$ <sup>+</sup> splenic and lymph node DCs (21) suggests a common origin.

Lymphoid and myeloid DCs differ in phenotype, localization, and function. Both subsets express high levels of CD11c, class II major histocompatibility complex (MHC), and the costimulatory molecules CD86 and CD40. To date, the most reliable marker to distinguishing these two subsets is CD8 $\alpha$ , which is expressed as a homodimer on the lymphoid DC, but is absent from the myeloid subset (19, 21–23). Other markers such as DEC-205 and CD1d are expressed at higher levels on lymphoid DCs, but they can be upregulated on myeloid DCs by *in vitro* culture (19, 21–23) or LPS treatment (B Pulendran et al, unpublished observations). Lymphoid DCs are localized in the T cell-rich areas of the periaarteriolar lymphatic sheaths (PALS) in the spleen and lymph nodes (17, 23–25). In contrast, myeloid DCs are in the marginal zone bridging channels of the spleen (17, 23–25) but can be induced to migrate to the PALS under the influence of proinflammatory signals such as LPS (24) or parasite extracts (26). The lymphoid

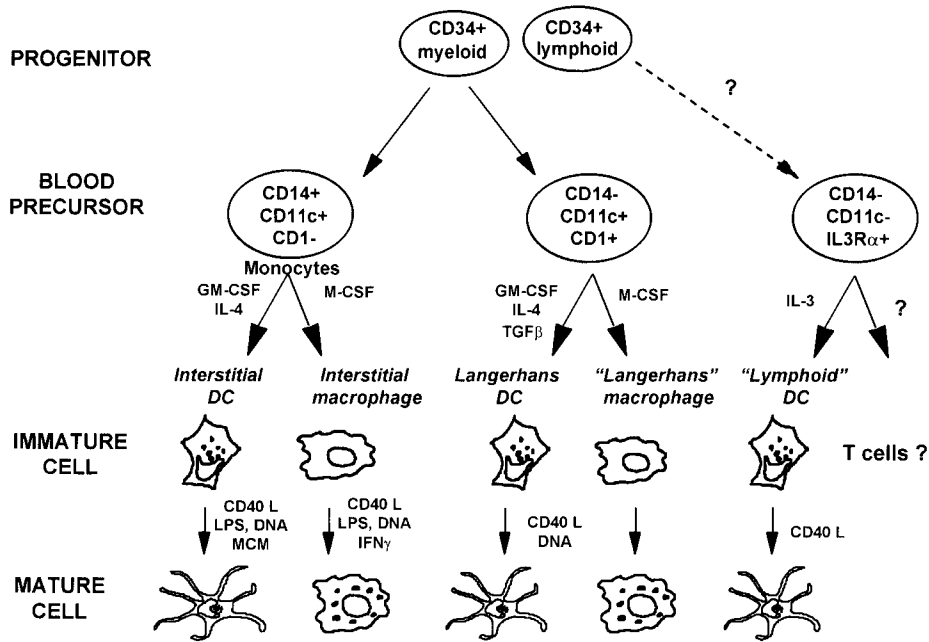
DCs make higher levels of interleukin (IL)-12 (23, 26–28) and are less phagocytic than myeloid DCs (23, 25). IL-12 induces production of IFN $\gamma$  in lymphoid but not in myeloid DCs (28). In vitro, the lymphoid DCs were reported to prime allogeneic CD4 and CD8 T cells less efficiently than myeloid DCs (29, 30). Although the interpretation could be that lymphoid DCs may downregulate T cell responses, the in vivo demonstration of this hypothesis is still pending. In vivo, both lymphoid and myeloid DCs appear to prime Ag-specific CD4<sup>+</sup> T cells efficiently (27, 31; see below).

Flt3 ligand (Flt3-L) and GM-CSF can expand mature DC in mice. Flt3-L targets primitive hematopoietic progenitors in the bone marrow, inducing their expansion and differentiation (32), and both lymphoid and myeloid DC numbers increase dramatically upon Flt3-L injection (22, 23, 33). Flt3-L treatment leads to an increase in DC numbers in multiple organs in mice, including spleen, lymph nodes, blood, thymus, Peyer's patch, liver, and lungs. In contrast, GM-CSF preferentially expands the myeloid DC subset in vivo (31).

In mice, the relationship between Langerhans cells (LCs) and lymphoid and myeloid DCs is not completely understood. LCs do share many common markers with myeloid DCs in the dermis (34). Studies of skin sensitization suggest that a subset of myeloid DC found in the draining lymph nodes represents LCs that have migrated there from the skin (35). LC development seems to be critically dependent on transforming growth factor (TGF)- $\beta$  because TGF- $\beta$  knockout mice are devoid of LCs, but not of their precursors (36). Genetic evidence for separate pathways of DC development comes from the study of mice that are deficient in certain genes. Thus RelB  $-/-$  mice are deficient in myeloid DCs; mice bearing a mutant Ikaros gene are deficient in lymphoid DCs (37–40).

## Humans

DC heterogeneity in humans is reflected at four levels. (a) *Precursor Populations*. For instance, in humans, at least two subsets of DC precursors circulate in the blood: CD14<sup>+</sup> CD11c<sup>+</sup> monocytes and lineage-negative (LIN<sup>neg</sup>) CD11c<sup>+</sup> IL-3R $\alpha$ <sup>+</sup> precursor DCs (41–44; Figure 2). The LIN<sup>neg</sup> CD11c<sup>+</sup> cells may represent a third precursor, although these cells are more committed because they can spontaneously differentiate into DCs when put into culture. (b) *Anatomical Localization*. The level of heterogeneity reflected by anatomical localization includes skin epidermal LCs, dermal (interstitial) DCs (intDCs), splenic marginal DCs, T-zone interdigitating cells, germinal-center DCs, thymic DCs, liver DCs, and blood DCs. Although certain phenotypic differences have been observed among these different DC subsets, their lineage origins, maturation stages, and functional differences have not been clearly established. (c) *Function*. Both murine and human DC subsets exert different functions, particularly in the regulation of B cell proliferation and differentiation of T cells toward type 1 or type 2, as discussed later. (d) *The Final Outcome of Immune Response*. The final outcome of immune response refers to the induction of tolerance or immunity.



**Figure 2** Three subsets of human dendritic cells (DC) and related macrophages. Myeloid  $CD34^+$  progenitors differentiate into monocytes ( $CD14^+ CD11c^+ DC$  precursors) that yield the immature DCs in response to granulocyte/macrophage colony-stimulating factor-positive (GM-CSF) interleukin (IL)-4 and macrophages in response to macrophage colony-stimulating factor (M-CSF) (the interstitial pathway). Myeloid progenitors also differentiate into  $CD11c^+ CD14^-$  precursors, which yield Langerhans cells in response to GM-CSF and IL-4 and transforming growth factor (TGF)  $\beta$ , and macrophages in response to M-CSF. Note that these later precursors can spontaneously differentiate into DCs in cultures. The  $CD14^- CD11c^- IL-3R\alpha^+$  DC precursor (also called pDC2, IFN $\alpha$ -producing cell, or plasmacytoid T cell; a possible equivalent to the murine lymphoid DCs) may originate from the lymphoid  $CD34^+$  progenitor. A blood cell population with a comparable phenotype has been shown to yield T cells in fetal thymic organ cultures.  $CD11c^- IL-3R\alpha^+$  DC precursors differentiate into immature DCs in response to IL-3. The immature cells differentiate to mature cells in response to cytokines (MCM, monocyte-conditioned medium) or pathogen products [lipopolysaccharide (LPS) or DNA].

A large body of literature has recently accumulated concerning the origin and in vitro differentiation pathways of DCs (9, 10, 45). Overall, lymphoid and myeloid DCs have been characterized, although the existence of human lymphoid DCs is somewhat controversial (46, 47). The  $CD11c^+ CD14^+$  monocytes (48–50), as well as  $LIN^{neg} CD11c^+$  blood DCs (51) give rise to immature DCs under the influence of GM-CSF and IL-4 or tumor necrosis factor (TNF). Furthermore,  $CD11c^+$  blood DCs can differentiate into LCs in the presence of TGF- $\beta$  (51). In

fact, TGF- $\beta$  appears to represent a major factor for LC differentiation both in mice and humans (36, 52). Both monocytes and CD11c<sup>+</sup> blood DCs can give rise to macrophages if cultured with GM-CSF or M-CSF, suggesting the plasticity of the DC system (53, 54). Distinct factors regulate the survival and differentiation of CD11c<sup>-</sup> DC precursors, originally described as plasmacytoid T cells or plasmacytoid monocytes (41, 42, 55–58). These cells die rapidly after isolation and are critically dependent on IL-3 for survival and CD40-L for maturation. Phenotypically similar populations of adult blood cells have been shown to express the pre-T cell receptor and to contain precursors of mature CD4<sup>+</sup> TCR $\alpha/\beta$ <sup>+</sup> T cells, hence the presumption of the lymphoid origin of CD11c<sup>-</sup> DC (59). Monocytes and CD11c<sup>-</sup> IL-3R $\alpha$ <sup>+</sup> DC precursors display many phenotypic differences, and monocytes, but not CD11c<sup>-</sup> DC precursors, express significant levels of CD11b, CD13, CD14, CD33, and CD45RO. Whereas monocytes express high GM-CSFR $\alpha$  and low IL-3R $\alpha$ , CD11c<sup>-</sup> DC precursors display reciprocal patterns of cytokine receptor expression, low GM-CSFR $\alpha$ , and high IL-3R $\alpha$ , consistent with in vitro cytokine responsiveness. Finally, immature monocyte-derived DCs display high endocytic/phagocytic capacity, contrary to immature CD11c<sup>-</sup> DCs.

The generation of intDCs (60) and LCs from CD34<sup>+</sup> hematopoietic progenitors is regulated by the same cytokines that drive differentiation of blood precursors (53, 61, 62). Whereas the corresponding mature DC progeny are equally potent in stimulating the proliferation of naive T cells, only intDCs induce the IL-2-driven differentiation of naive B cells in vitro (62). Although both subsets express IL-12 upon CD40 ligation, intDCs exclusively express IL-10 (63). Finally, intDCs demonstrate a high efficiency of Ag capture, that is ~10-fold higher than that of LCs. intDCs also express high levels of nonspecific esterases, whereas LCs do not. Currently, no biological function specific to LCs has been formally identified. LCs lack functional mannose receptors and are poor stimulators of Ag-specific CD4<sup>+</sup> T cell clones when compared with monocyte-derived DCs (64). This characteristic, together with observations that CD34<sup>+</sup> HPC<sup>-</sup> derived DCs, composed of both LCs and intDCs, are more potent in the priming of Melan-A/MART-1-specific cytotoxic T lymphocytes (CTLs) than DCs generated from monocytes (65), prompts the hypothesis that the primary function of LCs may be the priming of CD8<sup>+</sup> T cells. Our current view of the differentiation pathways and maturation stages of DCs and their precursors is summarized in Figure 2.

Besides replenishing the pool of tissue-residing immature DCs, circulating DC precursors play a critical role in the immediate reaction to pathogens and in the shaping of immune response. Monocytes have long been recognized as initial effectors of LPS-related inflammatory responses, as well as a limited source of IFN $\alpha$ . The exact nature of IFN $\alpha$ -producing cells has been, however, enigmatic until the recent identification of CD11c<sup>-</sup> IL-3R $\alpha$ <sup>+</sup> blood DC precursors as a major source of IFN $\alpha$  in response to virus (55, 56, 58, 66). The emerging finding is again the plasticity of the system, illustrated by (a) the specialization of DC precursors to respond to different pathogens, virus, or bacteria; and (b) the dual

function of these cells at two distinct stages of differentiation, as exemplified by (i) the ability of precursor DCs to secrete large amounts of proinflammatory and/or antiviral cytokines and (ii) ability of mature DCs to activate and modulate T cell responses. Thus, to efficiently combat pathogen invasion, DCs link the two branches of the immune system: the Ag-nonspecific innate immunity and the Ag-specific adaptive immunity (Figure 1).

## INTIMATE LINK BETWEEN ANTIGEN CAPTURE, MIGRATION, AND MATURATION

An important attribute of DCs at various differentiation stages is their mobility (67). DCs migrate from bone marrow to peripheral tissue, where their encounter with Ags triggers their migration to the secondary lymphoid organs. There, Ag-bearing DCs select the Ag-specific lymphocytes from the pool of recirculating T cells. The selective migration of DCs and their residence in nonlymphoid as well as lymphoid organs are tightly regulated events whose molecular control is being rapidly unraveled (Figure 3, see color insert).

### Recruitment of Dendritic Cell Precursors

Newly generated DCs migrate, presumably through the blood stream, from the bone marrow to nonlymphoid tissues, where they eventually become resident cells. DCs accumulate rapidly (within an hour) at the sites of Ag deposition as demonstrated in bronchial epithelium after Ag inhalation (68, 69). This accumulation likely represents recruitment of circulating DC precursors, in response to the production of chemokines upon local inflammation. In vitro, immature DCs respond to a large spectrum of chemokines through specific receptors (Table 1). Different DC subsets display unique sensitivity to certain chemokines. For instance, CD34<sup>+</sup> HPC-derived immature DCs express CCR6, whose ligand, MIP-3 $\alpha$  (also identified as LARC, Exodus-1), appears to be the most powerful chemokine guiding their migration (70, 71). However, MIP-3 $\alpha$  has no effect on monocyte-derived immature DCs (70–72), a difference that may be linked either to a putative inhibitory effect of IL-4 on CCR6 expression or to a specific activity of MIP-3 $\alpha$  on LCs present within CD34<sup>+</sup> HPC-derived DCs. MIP-3 $\alpha$  expression is restricted to epithelium, as observed in tonsils and gut. Its induction during inflammatory processes (70, 73) might represent a fundamental mechanism for the chemoattraction of immature LCs or their precursors to inflammatory epithelial sites. In this context, MIP-3 $\alpha$  displays selective activity for other leukocyte populations (memory T cells,  $\gamma/\delta$  T cells) with skin or gut epithelial tropisms (73, 74). The accumulation of immature DCs, mostly LCs, in the breast carcinoma bed is also associated with the production of MIP-3 $\alpha$  by tumor cells (75).

During their migration, DCs are involved in several adhesion events. For instance, E-cadherin, uniquely expressed by LCs, permits, through homotypic

**TABLE 1** Expression of chemokine receptors by dendritic cells<sup>a</sup>

Receptor	Ligands
Immature DC	
CCR1	MIP-1 $\alpha$ , RANTES, MCP-3, MIP-5
CCR2	MCPs
CCR4	TARC, MDC
CCR5	MIP-1 $\alpha$ , MIP-1 $\beta$ , RANTES
CCR6 (LC only)	MIP-3 $\alpha$
CXCR1	IL-8
CXCR4	SDF-1
Mature DC	
CCR7	MIP-3 $\beta$ , SLC (6Ckine)

<sup>a</sup>Abbreviations: DC, dendritic cells; MIP, macrophage inflammatory protein; RANTES, regulated on activation, normal T expressed and secreted; MCP, monocyte chemoattractant protein; TARC, thymus and activation-regulated chemokine; MDC, monocyte derived chemokine; SDF, stromal derived factor; IL, interleukin; SLC, secondary lymphoid-tissue chemokine.

interactions, the residence of LCs in epidermis (75, 76). Ag encounter results in downregulation of E-cadherin that allows LC migration out of the skin (77). The release of type IV collagenase by LCs may facilitate their migration through the basement membranes (78). Likewise, human macrophage elastase, which degrades several components of the extracellular matrix, is highly expressed by DCs and may thus contribute to their migration (S Lebecque, unpublished observation). Analysis of 17,000 genes from a complementary-DNA library constructed from immature monocyte-derived DCs identified the expression of many genes presumably involved in cell migration, including a metalloproteinase with elastolytic activity as well as a DC-specific *HAI-2* gene, a serine protease inhibitor of hepatocyte growth factor activator (79). Overall, the differentiation of monocytes to DCs was accompanied by significant changes in the expression of genes related to cell structure and motility.

## Antigen Capture

Immature DCs are very efficient in Ag capture and can use several pathways, such as (a) macropinocytosis; (b) receptor-mediated endocytosis via C-type lectin receptors (mannose receptor, DEC-205) (64, 80–84) or Fc $\gamma$  receptor types I (CD64) and II (CD32) [uptake of immune complexes or opsonized particles (85)]; and (c) phagocytosis of particles such as latex beads (86), apoptotic and necrotic cell fragments (involving CD36 and  $\alpha$ v $\beta$ 3 or  $\alpha$ v $\beta$ 5 integrins) (87–89), viruses, and bacteria including mycobacteria (90, 91), as well as intracellular parasites such as *Leishmania major* (92). DCs can also internalize the peptide loaded heat shock proteins gp96 and Hsp70 through presently unknown mechanisms (93, 94).



Whereas mannosylated Ags are rapidly internalized by intDCs and selectively targeted to a class II processing/presentation pathway, the capacity of LCs to capture mannosylated Ags is somewhat controversial. Freshly isolated murine epidermal LCs can uptake both mannosylated and nonmannosylated Ags (84). However, human LCs lack classical mannose receptors and have poor endocytic capacity and low levels of lysosome markers (64). A novel C-type lectin (called Langerin), recognized by LC-specific monoclonal antibody DCGM4 and seemingly involved in the formation of Birbeck granules, has been recently cloned (95, 96). These differences between LCs and intDCs extend to differential expression of Fc $\epsilon$  (97) and Fc $\gamma$  receptors, further strengthening the notion of DC functional heterogeneity and its effect on the type of immune response induced.

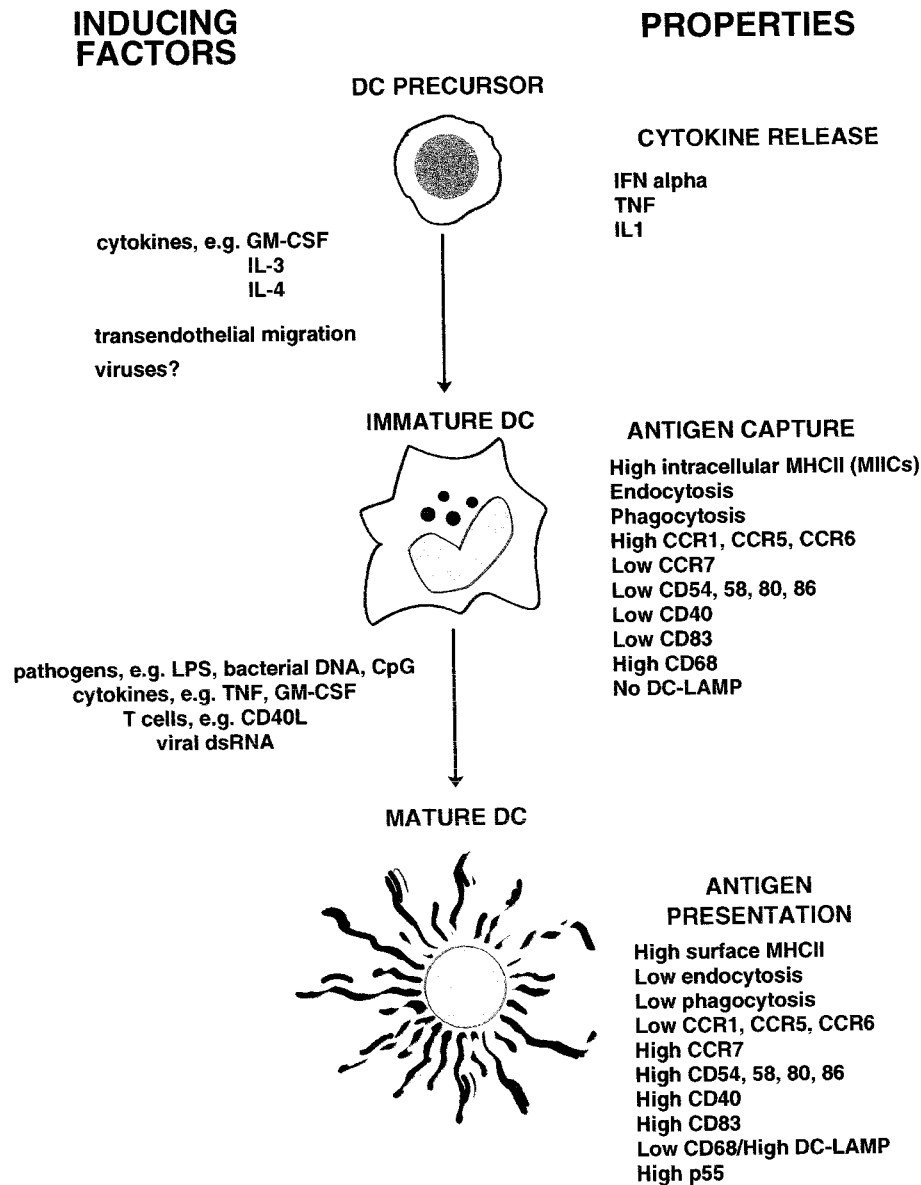
## Migration to Draining Lymphoid Organs and Maturation

The antigen/pathogen induces the immature DC to undergo phenotypic and functional changes that culminate in the complete transition from Ag-capturing cell to APC. DC maturation is intimately linked with their migration from the peripheral tissue to the draining lymphoid organs, and therefore these two key events in the life span of DCs are discussed together.

***Dendritic-Cell Activation and Maturation*** Several molecules including CD40, TNF-R, and IL-1R have been shown to activate DCs and to trigger their transition from immature Ag-capturing cells to mature Ag-presenting DCs. DC maturation is a continuous process initiated in the periphery upon Ag encounter and/or inflammatory cytokines and completed during the DC-T cell interaction. The molecules involved in T cell-mediated DC maturation are discussed later.

Numerous factors induce and/or regulate DC maturation (Figure 4), including (a) pathogen-related molecules such as LPS (91), bacterial DNA (98–100), and double-stranded RNA (101); (b) the balance between proinflammatory and anti-inflammatory signals in the local microenvironment, including TNF, IL-1, IL-6, IL-10, TGF- $\beta$ , and prostaglandins; and (c) T cell-derived signals. The maturation process is associated with several coordinated events such as (a) loss of endocytic/phagocytic receptors; (b) upregulation of costimulatory molecules CD40, CD58, CD80, and CD86; (c) change in morphology, (d) shift in lysosomal compartments with downregulation of CD68 and upregulation of DC-lysosome-associated membrane protein (DC-LAMP, as discussed later); and (e) change in class II MHC compartments.

Morphological changes accompanying DC maturation include a loss of adhesive structures, cytoskeleton reorganization, and acquisition of high cellular motility (102). An important controller of cytoskeleton remodeling may be the actin-bundling protein p55 fascin, expressed at high levels in blood DCs and in interdigitating DCs located in the T cell areas of lymph nodes (103). Indeed, the formation of dendritic projections in maturing LCs can be inhibited by fascin antisense oligonucleotides (104). DC cytoskeleton abnormalities and reduced



**Figure 4** Maturation of dendritic cells (DCs). The left side of the scheme shows the factors inducing progression from one stage to another (GM-CSF, granulocyte/macrophage colony-stimulating factor; IL, interleukin; LPS, lipopolysaccharide; TNF, tumor necrosis factor; dsRNA, double-stranded RNA); the right side shows the main properties of each differentiation/maturation stage (IFN, interferon; MHCII, major histocompatibility complex II; MIIC, MHCII-rich compartment; LAMP, lysosome-associated membrane protein).

mobility in vitro have been detected in Wiskott-Aldrich syndrome (WAS) (105), an X-linked recessive disorder characterized by thrombocytopenia, eczema, and immunodeficiency. The WAS gene encodes a 502-amino-acid proline-rich protein (WASp) whose transcripts are detectable throughout differentiation from early hematopoietic progenitors to DCs (106). Because the Cdc42/Rac-binding motif of WASp can control cytoskeleton rearrangement, this molecule may be of importance during DC capture of pathogens and establishment of DC–T cell contacts.

Among molecules regulating DC activation/maturation, DC immunoreceptor is a calcium-dependent (C-type) lectin (S Lebecque, manuscript in preparation) that displays an intracellular domain containing an immunoregulatory tyrosine-based inhibitory motif (ITIM) characteristic of immunoglobulin superfamily members and membrane lectins (107, 108).

**Migration of Antigen-Bearing Dendritic Cells** Allogeneic skin transplantation models (109–111), as well as injection of labeled DCs (112) or *Leishmania*-infected LCs (92) demonstrated that DCs leave the nonlymphoid organs through the afferent lymph. Pathogen products such as LPS and the local production of TNF $\alpha$  or IL-1 (113), all mediators of DC maturation, trigger peripheral DC migration into the T cell area of lymphoid organs. This migration of maturing DCs also involves a coordinated action of several chemokines. After Ag uptake, inflammatory stimuli turn off the response of immature DCs to MIP-3 $\alpha$  (and other chemokines specific for immature DCs) through either receptor downregulation or receptor desensitization dependent on autocrine chemokine production (70, 114, 115). Consequently, maturing DCs escape from the local gradient of MIP-3 $\alpha$ . Upon maturation DCs upregulate a single known chemokine receptor, CCR7 (116), and accordingly acquire responsiveness to MIP-3 $\beta$  (ELC, Exodus 3) and 6Ckine [secondary lymphoid-tissue chemokine (SLC), Exodus 2] (70, 117). Consequently, maturing DCs will leave the inflamed tissues and enter the lymph stream, potentially directed by 6Ckine that is expressed on lymphatic vessels (118, 119). Mature DCs entering the draining lymph nodes will be driven into the paracortical area in response to the production of MIP-3 $\beta$  and/or 6Ckine by cells spread over the T cell zone (70, 120). The newly arriving DCs might themselves become a source of MIP-3 $\beta$  and 6Ckine (70, 114, 120), allowing an amplification and/or a persistence of the chemotactic signal. Because these two chemokines can attract mature DCs and naive T lymphocytes (118, 120, 121), they are likely to play a key role in helping Ag-bearing DCs to encounter specific T cells. The role of MIP-3 $\beta$  and 6Ckine is supported by a natural mutant mouse for 6Ckine (SLC) (122–124) and CCR7-deficient mice, both of which have a specific deficiency in T cell and DC homing into lymph nodes (125). Upon encounters with T cells, which can take place not only in the secondary lymphoid organ but also at the site of tissue injury, DCs receive additional maturation signals from CD40 ligand, RANK/TRANCE, 4–1BB, and OX40 ligand molecules, which induce the release of chemokines such as IL-8, fractalkine (126), and macrophage derived chemokines that attract lymphocytes (127, 128).

## ANTIGEN PROCESSING AND PRESENTATION

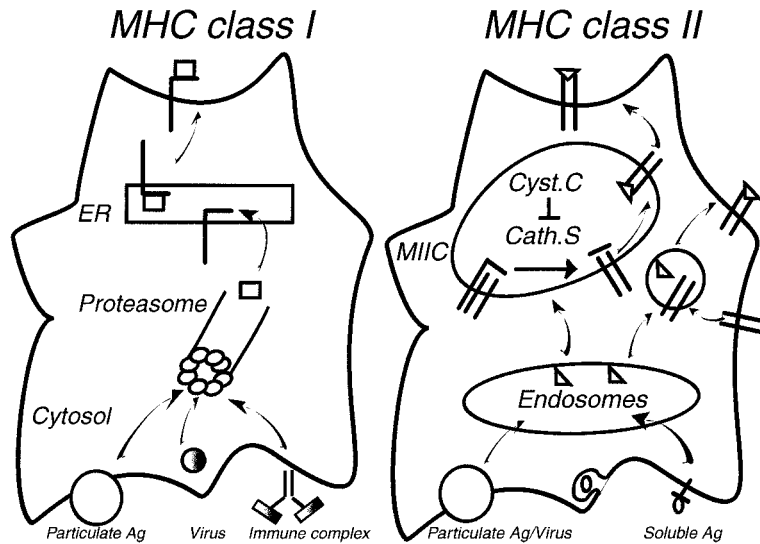
DCs are well equipped to capture and process Ags, and a number of molecules involved in these processes have been identified and are discussed below. Furthermore, evaluation of 3000 sequences randomly cloned out of a library constructed from CD34-derived DCs showed that 20% of the sequences were related to Ag presentation (MHC molecules constituting 7%), and 6% of sequences constituted enzymes including several members of the serine- and metalloprotease families (S Lebecque unpublished observations).

### Major Histocompatibility Complex Class II

Soluble and particulate Ags are efficiently captured by immature DCs and targeted to MHC class II compartments (49, 80, 83, 129, 130; Figure 5). Immature DCs constantly accumulate MHC class II molecules in lysosome-related intracellular compartments identified as MHC class II-rich compartments (MIICs), with multivesicular and multilamellar structure (131, 132). The captured Ag is directed towards MIICs containing HLA-DM that promotes the catalytic removal of class II-associated invariant chain peptide and enhances peptide binding to MHC class II molecules (133, 134). However, the loading of class II Ags within DC can also occur in the absence of the invariant chain (135). In immature DCs, Ags and macromolecules gain access to mildly acidic prelysosomal MIICs (136), where MHC class II-Ii chain complexes accumulate. The proteolytic degradation of Ii is regulated by the ratio between cathepsin S and its endogenous inhibitor cystatin C (137). Upon maturation, cystatin C is downregulated, and the activity of cathepsin S increases, promoting Ii degradation and allowing the export of peptide-loaded class II molecules to the cell surface.

Whereas, in immature DCs, class II molecules are rapidly internalized and have a short half-life, maturation/inflammatory stimuli lead to a burst of class II synthesis and translocation of the MHC II-peptide complexes to the cell surface where they remain stable for days and are available for recognition by CD4<sup>+</sup> T cells (102, 130, 138, 139). DC-LAMP, a novel marker exquisitely induced in mature DCs, is localized in lysosomes as well as in MHC class II compartments immediately before the translocation of MHC class II molecules to the cell surface (140), which is coordinated with DC maturation. It is interesting to note that IL-10 can block translocation of peptide-class II complexes to DC plasma membrane, in parallel with inhibition of DC maturation (141).

The different surface receptors used by DC subsets to capture Ags, as well as subtle differences in proteolytic machinery, may determine the nature of immunodominant peptides presented by MHC class II molecules (142). These variations in Ag processing may permit recruitment of CD4<sup>+</sup> T cells with diverse TCR specificities and spreading of the response.



**Figure 5** Dendritic cells load their major histocompatibility complex (MHC) molecules in multiple ways. (*Left*) MHC class I. Besides the classical endogenous pathway that loads peptides from self and intracellular pathogens, dendritic cells can also load MHC class I antigens through exogenous pathways with peptides originating from phagocytosed particulate antigens or immune complexes. Peptides are generated in the proteasome, transferred into the endoplasmic reticulum (ER), and loaded onto the nascent MHC class I molecules. (*Right*) MHC class II. Dendritic cells capture soluble antigen (Ag) either through macropinocytosis or receptor-mediated endocytosis. They also capture particles through phagocytosis. The antigens are subsequently degraded in endosomes, and the generated polypeptides are transported into the MHC class II-rich compartments (MIIC) for their loading onto the nascent MHC class II molecules while DCs mature. The invariant chain, associated with nascent MHC class II, is cleaved by cathepsin S (Cath.S), which in immature DCs is inhibited by cystatin C (Cyst.C). After maturation, cystatin C is down-regulated, thereby releasing active cathepsin S. The HLA-DM molecules help the loading of peptides onto MHC class II molecules. A fraction of the peptides are loaded onto empty MHC class II molecules recycled from the cell surface (cycle on the right).

## Major Histocompatibility Complex Class I

To generate CD8<sup>+</sup> cytotoxic killer cells, DCs present antigenic peptides on MHC class I molecules, which can be loaded through both an endogenous and an exogenous pathway (143, 144; Figure 5).

**Endogenous Major Histocompatibility Complex Class I Pathway** The endogenous MHC class I pathway operates through the degradation of cytosolic proteins and the loading of peptides onto newly synthesized MHC class I molecules within the endoplasmic reticulum. Ag processing occurs first in the cytosol through an

ATP-dependent proteolytic system, which starts by ubiquitin conjugation. DCs, similarly to B cells, constitutively express di-ubiquitin, which could permit more efficient Ag processing (145). This gene, also known as *FAT10* (146), encodes a di-ubiquitin protein containing tandem head to tail ubiquitin-like domains, with the conservation of key functional residues. The ubiquitinated proteins are directed to the proteasome, which cleaves the protein into peptides. The peptides are then translocated into the ER via ATP-dependent TAP1/2 transmembrane transporters and are trimmed into 8–10 mers, which accommodate the MHC class I-binding groove.

**Cross-Priming and Class I Presentation of Exogenous Antigens** Over 20 years ago Bevan observed priming of host MHC class I-restricted CTLs for minor Ags after immunization with cells that lacked the cognate MHC class I molecules (147, 148). This property, termed cross-priming, suggested that minor Ags could be transferred to host cells for presentation by host MHC class I molecules (149). This observation and many others have led to the conclusion that DCs and, to a lesser extent, macrophages have an alternative MHC class I pathway that can present peptides derived from extracellular Ags. Two routes for the exogenous MHC class I pathway have been described, a TAP-independent pathway in which Ag is most likely hydrolyzed in endosomes (150) and a phagosome-to-cytosol pathway (151, 152) that is TAP dependent. This pathway is thought to be involved in immune responses against transplantation Ags (147), particulate Ags (151), tumors (153), and viruses (154). It is also operative in the development of tolerance (155). The engulfment and processing of cell bodies by DCs represent a possible pathway for the loading of MHC class I (87, 88, 156). Indeed, monocyte-derived DCs loaded with apoptotic bodies obtained from either macrophages or HeLa cells infected with influenza virus stimulate the proliferation of influenza-specific T cells and the generation of class I-restricted influenza-specific CD8<sup>+</sup> CTLs (88). Most recently, FcγR-mediated capture of immune complexes (157) and exosomes derived either from tumor cells or from tumor-peptide-pulsed DCs (158) were demonstrated as another pathway permitting access to DC MHC class I presentation. Finally, transfer of peptides carried by heat shock proteins, including hsp70 and gp96, allows in vivo development of protective immunity and tumor rejection in murine models (94, 159). Thus, manipulation of the exogenous class I presentation pathway may permit priming of T cells with desired Ag specificity, for instance in tumor immunotherapy.

## CD1 Molecules

Recent studies have identified the CD1 family as nonclassical, Ag-presenting molecules involved in regulation of T cell responses to microbial lipids and glycolipids-containing Ag (for reviews, see 160, 161). Both endogenous and exogenous lipids can be presented, and this pathway may contribute not only to microbial immunity but to autoimmunity and antitumor responses. CD1 mole-

cules, a hallmark of the DC phenotype, constitute a family of  $\beta 2$ -microglobulin-associated nonpolymorphic glycoproteins that assemble with a nonprocessed Ag in the endosomal/lysosomal compartments and present Ag in a TAP-independent manner. In humans, four CD1 proteins (CD1a–d) are expressed by myeloid DCs, whereas in mice only CD1d has been identified. CD1 proteins are functionally heterogeneous, and two subgroups can be identified. Subgroup I, including human CD1b–c, can present glycolipids to a large repertoire of T cells. Indeed, mycobacteria-specific, CD1b-restricted  $CD8^+ \alpha/\beta$  TCR T cells have been demonstrated. Binding of the lipids to these CD1 molecules requires endosomal acidification. Subgroup II includes mouse and human CD1d and binds a limited set of Ags ( $\alpha$ -galactosylceramide) and activates a restricted set of T cells as well as NK T cells (162). CD1-restricted presentation appears to also regulate  $\gamma/\delta$  T cells and intestinal intraepithelial lymphocytes. Much remains to be learned about this presentation pathway and the possibilities of its use in vaccine protocols.

## ANTIGEN PRESENTATION AND T CELL ACTIVATION

### T Cell Priming

The ability to prime naïve  $CD4^+$  T cells constitutes a unique and critical function of DCs both in vitro and in vivo. Soluble Ag-pulsed DCs elicit potent Ag-specific T-helper responses when injected into mice (163). Demonstration of DC–T-helper-cell interactions in the PALS by immunohistology suggests direct Ag presentation by pulsed DCs (164). However, an alternative indirect pathway may exist whereby apoptotic fragments of exogenous DCs can be phagocytosed, processed, and presented by resident DCs in the PALS (129). The relative contribution of both Ag-presentation pathways to T cell priming in vivo remains to be investigated. In the presence of free Ag, T-helper cells primed by DCs can interact with B cells and stimulate Ag-specific antibody production (165). The extent of  $CD4$  T cell and antibody responses can be dramatically enhanced in vivo by increased DC numbers, as shown recently with Flt3-L (166). Furthermore, the potent immunogenicity of DCs can result in the abrogation of peripheral T cell tolerance against soluble Ags (166), viral Ags (167), tumors (168), and transplant Ags (169), as well as in neonates (170).

DCs are equally important in priming naïve  $CD8^+$  T cells. In vitro, DCs can stimulate the proliferation of allogeneic  $CD8^+$  T cells (171), directly in the absence of T cell help (172, 173). They can also generate Ag-specific CTLs from naïve precursors (174–176). Strong CTL responses can be induced in vivo by injection of mice with Ag-bearing DCs, including (a) allogeneic DCs (177), (b) peptide-pulsed DCs (178), (c) protein-loaded DCs (179, 180), (d) DCs transfected with DNA (181), (e) DCs expressing virally encoded Ags (182, 183), and (f) DCs pulsed with RNA (184). Although DCs can activate  $CD8^+$  T cells directly (172, 185, 186), they often require  $CD4$ –T cell help. In traditional models of CTL

activation, the CD4<sup>+</sup> T cells and CD8<sup>+</sup> T cells were thought to recognize Ag on the same Ag-presenting cell. However, in the current model (187–189), the APCs are licensed to activate T-killer cells by T helpers via upregulation of CD40-L on the DCs. Thus, a conditioned DC becomes a temporal bridge between a CD4<sup>+</sup> T-helper cell and a T-killer cell. In addition to priming, DCs appear essential to maintain survival of naive CD4 T cells (190) and immune T cell memory (191).

## Molecules Involved in Dendritic-Cell/T Cell Interaction

It remains to be determined whether the unique ability of DCs to prime T cells results from the expression of molecules unique to DCs or from the high density of molecules involved in DC–T cell interactions. MHC products and MHC-peptide complexes are 10- to 100-fold higher on DCs than on other APCs like B cells and monocytes (130). Recognition of MHC-peptide complexes on DCs by Ag-specific TCRs constitutes “signal one” in DC–T cell interaction. DC–T cell clustering is mediated by several adhesion molecules, like integrins  $\beta 1$  and  $\beta 2$  and members of the immunoglobulin superfamily (CD2, CD50, CD54, and CD58) (9, 10). Recently, a high-affinity receptor for intercellular adhesion molecule 3 (with no homology to LFA1) was found specifically expressed on monocyte-derived DCs (Figdor, personal communication). The crucial factor, that constitutes “signal two,” required to sustain T cell activation, is the interaction between costimulatory molecules expressed by DCs and their ligands expressed by T cells. CD86 on DCs is so far the most critical molecule for amplification of T cell responses (192, 193).

T cells can activate DCs via CD40 ligand (CD40-L)-CD40 signaling leading to increased expression of CD80/CD86 and cytokine release (IL1, TNF, chemokines, and IL-12) (49, 187–189, 194). Triggering of CD40 on DCs results in upregulation of OX40 ligand (195), which then signals naive T cells to express IL-4 (196) and upregulates the chemokine receptor CXCR-5, whose ligand directs B lymphocytes into follicles (197). Accordingly, expression of the OX40-L transgene into DCs leads to accumulation of CD4 T cells in B follicles. Mature DCs also express 4-1BB ligand (198), which complements the function of OX40-L. 4-1BB is a costimulator expressed primarily on activated CD4<sup>+</sup> and CD8<sup>+</sup> T cells (199). 4-1BB costimulatory signals preferentially induce CD8<sup>+</sup> T cell proliferation and production of IFN $\gamma$  (but not of IL-4) (200), leading to the amplification of in vivo cytotoxic T cell responses in graft-vs-host disease as well as allograft rejection (201). Whether OX40-L and 4-1BB are expressed simultaneously or exclusively by the same DCs remains to be established. Engagement of RANK, a member of the TNFR family, by its ligand (RANKL/TRANCE) expressed on activated T cells, stimulates the secretion of cytokines like IL-1, IL-6, and IL-12 by DCs. This results in increased DC survival, by inhibition of DC apoptosis and, in turn, in enhanced proliferative T cell responses in mixed lymphocyte reactions. The demonstration that TRANCE is responsible for the CD40-



L-independent T-helper cell activation during viral infection suggests an important and specific role for this molecule during infection (202–204).

### Distinct Dendritic-Cell Subsets Elicit Distinct T Helper Cell Responses

DC subsets may provide T cells with the different cytokine/molecule microenvironments that determine the classes of immune response, for example, type 1 vs type 2 CD4 helper cell profile. In humans, monocyte-derived CD11c<sup>+</sup> DCs polarize naive T cells predominantly towards a Th1 profile, whereas the CD11c<sup>−</sup> DC subset induces T cells to predominantly produce Th2 cytokines (57). The extent of T cell polarization by CD11c<sup>−</sup> DCs may be related to their differentiation/maturation stages (55). Thus, CD11c<sup>−</sup> DC precursors may be prone to elicit more of the Th0 cytokine profile, whereas their mature progeny may induce Th2 differentiation. The induced pattern of T cell cytokine secretion is dependent on the DC production of IL-12 (205). Indeed, CD11c<sup>+</sup>, but not the CD11c<sup>−</sup> DC subset, can be induced to secrete IL-12 (57). Skewing is not restricted to CD4 T cells and also applies to CD8 T lymphocytes and NK T cells (206). In mice, the splenic CD8α<sup>+</sup> lymphoid DC subset primes naive CD4 T cells to make Th1 cytokines, whereas the splenic CD8α<sup>−</sup> myeloid DC subset primes naive CD4 T cells to make Th2 cytokines (27, 31). Consistent with this, GM-CSF, which preferentially mobilizes myeloid DCs in mice, elicits mainly IgG1 antibodies in response to soluble Ag, whereas Flt3-L, which mobilizes both lymphoid and myeloid DC subsets (31), also elicits IgG2a antibodies, a Th1 signature. DCs from IL-12-deficient mice fail to induce Th1 responses, suggesting the critical role of IL-12 in lymphoid DCs-induced Th1 responses (27). Lymphoid but not myeloid DCs can be induced to make large amounts of IL-12 (23, 26–28) and IFNγ (28). The mechanism by which myeloid DCs induce Th2 cytokines is not established, although IL-13 (207), IL-6 (208), and OX40-L (196) are good candidates. The involvement of CD80 and CD86 in Th1/Th2 polarization remains unclear, but, in some experimental systems, B7.1/CD80 was shown to promote Th1 responses, whereas B7.2/CD86 ligation tended to skew toward Th2 responses (209, 210). Overall, distinct DC subsets exist in mice and humans that differentially skew Th responses.

### DC Functions Exhibit Considerable Plasticity

Although it is clear that distinct DC subpopulations exhibit distinct functions, there is also evidence that these DC functions can be altered by the cytokine environment. In particular, DCs exhibit considerable plasticity in their ability to skew Th responses, and DCs that normally induce Th1 profiles can be converted to Th2-skewing cells when treated with anti-inflammatory cytokines such as IL-10 and TGFβ or with steroids (211) or prostaglandin E2 (212–217). In this context, DC subsets isolated from different organs differently affect Th responses. Thus,

mouse and rat DCs from Peyer's patches elicit Th2 responses, whereas those from spleen induce Th1 responses (218). Although the mechanisms underlying these functional differences are currently unknown, these observations may offer an explanation for the distinct immunological outcomes of oral vs systemic administration of Ags. Thus, adjuvants such as LPS or Flt3-L enhance immunological tolerance to orally administered Ags (219), but abrogate tolerance to systemic injections of Ags (166, 220).

DC plasticity is also reflected in their differentiation, which may determine the fate of Ag, that is, processing and presentation or degradation. This aspect is exemplified by the potential of macrophage to differentiate to DCs (54), a pathway that may permit high Ag capture (macrophage) and presentation (dendritic cell). The final signal for DC differentiation and maturation may be provided during the migration of DCs across endothelial barriers between the inflamed tissue and lymphatics (221, 222).

## Dendritic Cells and Tolerance

In mice, thymic DCs are capable of mediating negative selection of T cells in fetal organ cultures (223) and against superantigens *in vivo* (224, 225). In addition, thymic DCs can induce tolerance to myelin basic protein and limit the development of experimental autoimmune encephalomyelitis (226). The role of thymic DCs in negative selection (but not positive selection) was confirmed by targeted expression of MHC class II molecules on DCs (227). In the periphery, a role of DCs in establishing peripheral T cell tolerance has not yet been formally demonstrated. In fact, the available evidence suggests that DCs can abrogate T cell tolerance against soluble Ags (166), viral Ags (167), tumors (168), and transplant Ags (169) and in neonates (170). However, *in vitro* work from Shortman's group suggests that, in mice, both lymphoid and myeloid DCs can stimulate T cells, but that the lymphoid DC subset can limit the proliferation of T cells (29, 30). The lymphoid DCs appear to kill a proportion of the activated CD4<sup>+</sup> T cells (30), whereas they limit cytokine production of CD8<sup>+</sup> T cells (29). The relevance of these *in vitro* findings for the *in vivo* tolerance induction remains to be established. Finally, DCs are also considered to play an important role in the establishment of transplantation tolerance through the development of microchimerism (228–230).

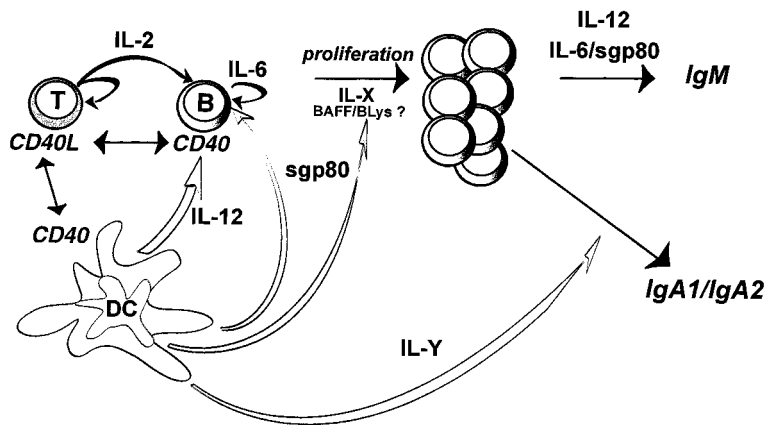
## REGULATION OF B LYMPHOCYTES

Beside activating naive T cells, DCs can directly activate naive and memory B cells. DCs enhance differentiation of CD40-activated memory B cells towards IgG-secreting cells through secretion of the soluble IL6R $\alpha$  gp80, which complexes to IL-6 (231, 232). DCs also help the differentiation of activated-naive B cells to plasma cells. This help is mediated by IL-12 in synergy with IL-6/soluble

IL6R $\alpha$ . DCs induce surface IgA expression on CD40-activated naive B cells, which is partially mediated by TGF $\beta$ , but neither IL-10 nor IL-12 appears to be involved (233). The presence of IgA switch circles in CD40-activated B cells cultured with DCs demonstrates the occurrence of DNA recombination. While DCs alone are able to induce CD40-activated naive B cells to express surface IgA, IL-10 is essential for further differentiation into IgA-secreting cells. Moreover, in the presence of IL10 and TGF $\beta$ , DCs skew CD40-activated naive B cells towards the secretion of both IgA1 and IgA2 subclasses (233). These results suggest the DC-mediated direct activation of naive B cell during the initiation of the immune response and the involvement of DC in the development of mucosal/humoral immune responses (Figure 6).

The germinal center (GC) is the microenvironment that allows the generation of B cell memory. There B cells proliferate and undergo somatic mutation, isotype switching, affinity selection, and differentiation into memory B cells or plasma-blasts. The GC also contains T cells, follicular DCs, and GC DCs (GCDCs). It is now clear that GCDCs are quite different from follicular DCs in phenotype and function (234, 235). GCDCs stimulate, in an IL-12-dependent manner, CD40-activated germinal-center B cell proliferation and drive their differentiation towards plasma cells. In addition, GCDCs induce IL10-independent isotype switching towards IgG1.

Thus, DC subsets have the capacity to directly regulate B cell responses. To generate a humoral immune response, Ag-specific CD4<sup>+</sup> T helper and Ag-



**Figure 6** Dendritic cells (DCs) directly signal B cells at the time of the “menage à trois.” CD40-activated DCs produce IL-X (BAFF/BLys?), which enhances the proliferation of CD40-activated B cells. CD40-activated DCs also secrete IL-12 and sgp80, which binds interleukin (IL)-6 (secreted by the B cells and some DCs). These factors, together with IL-2, induce CD40-activated naive B cells to differentiate into plasma cells secreting IgM. CD40-activated DCs also provide B cells the uncharacterized IL-Y, which together with IL-10 and TGF $\beta$  permits isotype switching towards IgA1 and IgA2.

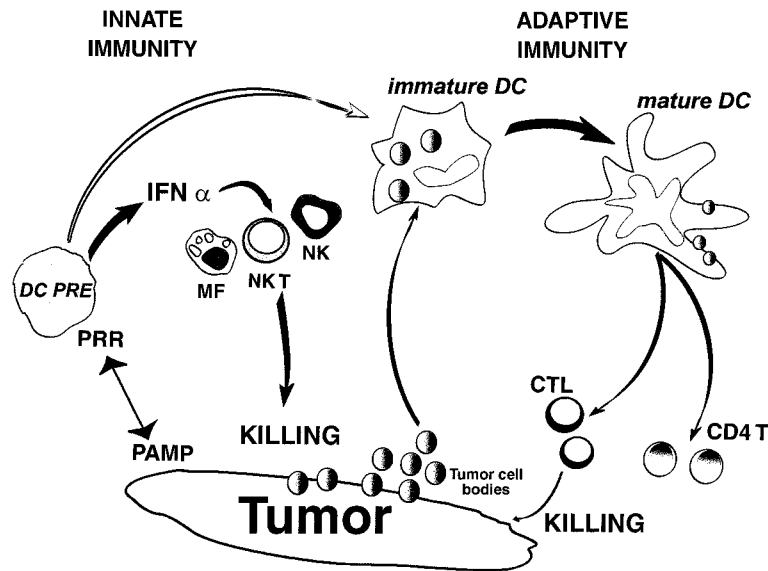
specific B cells must interact. Within paracortical areas of the secondary lymphoid organs, interdigitating DCs select the rare Ag-specific T and B cells. As recently demonstrated *in vivo* in the rat, DCs can also capture and retain unprocessed Ag, then transfer it to naive B cells to initiate a specific Th2-associated antibody response (236). This could be the role of the GCDC population localized within GCs and originally described as an “antigen-transporting cell” (237, 238), which could display the Ag to both T and B cells in a *ménage à trois*. However, one could consider that a conditioned DC can be a temporal bridge between a CD4<sup>+</sup> T helper and a B lymphocyte by analogy to recent models in which DCs offer costimulatory signals to CD4 helper T cells and CD8 T cells (187–189). During the extrafollicular reaction, interdigitating DCs could play a role in the induction of an IL-2–dependent IgM plasma cell differentiation. GC formation starts with the migration of GC founder cells in the follicles and involves Th2 CD4<sup>+</sup> T cells. CD40 activation upregulates OX40-L expression on DC and B cells (117, 195, 239) and early OX40 ligation promotes Th2 cytokine secretion (196) and causes CD4 T cell migration within B cell follicles (197). Thus, GCDC may contribute to the GC reaction and the role of OX40–OX40-L needs to be analyzed.

A novel member of the TNF family, designated BAFF/Blys-L (for B cell-activating factor belonging to the TNF family) and found on DCs and T cells, binds to a receptor restricted to B cells (185, 240, 241) and induces both proliferation and immunoglobulin secretion by different B cell subsets. BAFF may represent an important costimulator through which DCs regulate B cell proliferation and function. Like TNF $\alpha$  and FasL, BAFF/Blys is a transmembrane molecule that is processed and secreted by a protease yet to be identified. Decysin, a novel disintegrin-metalloproteinase isolated from GCDC and specific to mature DCs, represents a candidate for the cleavage of molecules of the TNF family and may thus play an important role in the regulation of T- and B cell functions (242).

## DENDRITIC CELLS AND EFFECTORS OF INNATE IMMUNITY

DCs at different stages of differentiation can regulate effectors of innate immunity such as NK cells and NK T cells. Both direct cell-cell interactions and indirect cytokine-mediated interactions have been implicated (Figure 7). Precursors of CD11c<sup>+</sup> DCs may activate NK cells through the release of IFN- $\alpha$ , thereby leading to enhanced antiviral and antitumor activity of NK cells (56, 58, 138). DCs at later stages of differentiation may regulate the activity of NK/NK T cells through the release of IL-12, IL-15 and IL-18 (243, 244).

Both murine and human NK T cells produce high amounts of IL-4 or IFN- $\gamma$  and thus may determine the type of induced immune responses. On recognition of the  $\alpha$ -galactosylceramide ( $\alpha$ -GalCer)/CD1d complex, NK T cells release IFN- $\gamma$  (162). However, DC subsets differentially regulate NK T cytokine profiles, with



**Figure 7** Dendritic cells as a link between innate immunity and adaptive immunity in antitumor immune responses (a hypothesis). Precursor DCs recognize tumor pathogen-associated molecular patterns (PAMP) through their pattern recognition receptors (PRR). Consequently, DCs release interferon (IFN)  $\alpha$ , which activates macrophages (MF), natural killer (NK) T cells, and NK cells that kill tumors leading to the release of tumor cell bodies. The cell bodies are captured by immature DCs (which may be the progeny of the initial precursor), which will mature and display tumor antigens for selection of tumor-specific lymphocytes. CD8<sup>+</sup> T cells will further directly kill the tumor while selected CD4 T cells will activate macrophages, NK cells, and eosinophils as discussed in Figure 1. The tumor may affect this process at various stages, by either preventing DC maturation or skewing the T cell responses towards the type 2.

monocyte-derived DCs promoting IFN- $\gamma$  release, whereas plasmacytoid DCs polarize NK T cells to IL-4 production (206). Because  $\alpha$ -GalCer or related glycolipids are expressed in bacteria and tumors and because tumor-protective responses can be induced by  $\alpha$ -GalCer-activated NK T cells, NK T cells may constitute an important effector mechanism of innate immunity (245).

NK cells can also be activated, directly or indirectly, by DCs (246, 247). In murine tumor models, DC transfer or in vivo mobilization with FLT3-L resulted in the NK-mediated rejection of MHC class I-negative tumors. Thus, it is likely that in vivo expansion of both NK cells and DCs by FLT3-L may account for the potent antitumor activity of FLT3-L (248, 249). This synergism could also apply to viral infections or transplantation. DCs can trigger NK cells, through the release of IL-12, to CD28-dependent and -independent cytotoxicity (250). This may result in elimination of B7-expressing cells, including autologous DCs, thus permitting either downregulation of response or amplification of the response via

cross-presentation of Ags released from dying DCs. Finally, activated NK cells may also elicit positive regulatory signals towards immature DCs, by promoting DC maturation in the spleen marginal zones (244, 249).

## DENDRITIC CELLS IN TUMOR IMMUNOLOGY

The immune system has the potential to eliminate neoplastic cells, as evidenced by occasional spontaneous remissions in renal-cell carcinomas and melanomas (9, 251, 252). Perhaps the most compelling evidence of active *in vivo* tumor-related immune responses arises from the study of paraneoplastic neurologic disorders that led to the discovery of onconeural Ags (253, 254). Paraneoplastic neurologic disorders are a rare group of neuronal degenerative diseases that develop as remote effects of systemic malignancies (253, 254). The discovery of onconeural antibodies led to the proposal that paraneoplastic cerebellar degeneration, associated with breast and ovarian cancer, is an autoimmune disorder mediated by the humoral arm of the immune system. These antibodies permitted the cloning of the cdr2 Ag, a protein with a coil/leucine zipper domain. Furthermore, the presence of cdr2-specific CD8<sup>+</sup> CTLs circulating in the blood of these patients has been demonstrated (88). The list of onconeural Ags is growing (254).

The induction of tumor immunity can be initiated by the effectors of innate immunity and further developed by cells of adaptive immunity, with DCs playing a central regulatory role (Figure 7). Several steps are involved, including (*a*) recognition of tumor molecules by DC precursors, (*b*) direct and IFN- $\gamma$ -mediated killing of transformed cells by NK/NK T cells activated by DCs, (*c*) capture and cross-presentation of released-tumor-associated Ags (TAAs) by immature DCs, (*d*) selection and activation of TAA-specific T cells, as well as nonspecific effectors including macrophages and eosinophils, and (*e*) homing of TAA-specific T cells to the tumor site and recognition of restriction elements leading to the elimination of tumor cells. Tumors may escape immune surveillance owing to alterations at each of these steps (9, 252). Thus, by release of cytokines such as IL-6, IL-10, M-CSF, and vascular endothelial growth factor, tumors can prevent DC differentiation and/or APC function (255). Indeed, tumor-associated DCs are usually of a low allostimulatory capacity, particularly if isolated from the progressing metastatic lesions, as in malignant melanoma, or from blood, as in patients with advanced breast cancer. Furthermore, IL-10 is capable of converting DC-APC function to the induction of Ag-specific anergy, thus leading to the state of tolerance against tumor tissue (256–258).

Analysis of tumor tissue distribution of DCs in breast carcinoma revealed two levels of heterogeneity: (*a*) immature CD1a<sup>+</sup> DCs, mostly of the LC type (Langerin<sup>+</sup>) are retained within the tumor bed in >90% samples, (*b*) mature DCs—CD83<sup>+</sup> DC-LAMP<sup>+</sup> DCs present in 60% of samples—are confined to peritumoral areas. The high numbers of immature DCs found in the tumor may best be explained by high levels of MIP3 $\alpha$  expression by virtually all tumor cells,

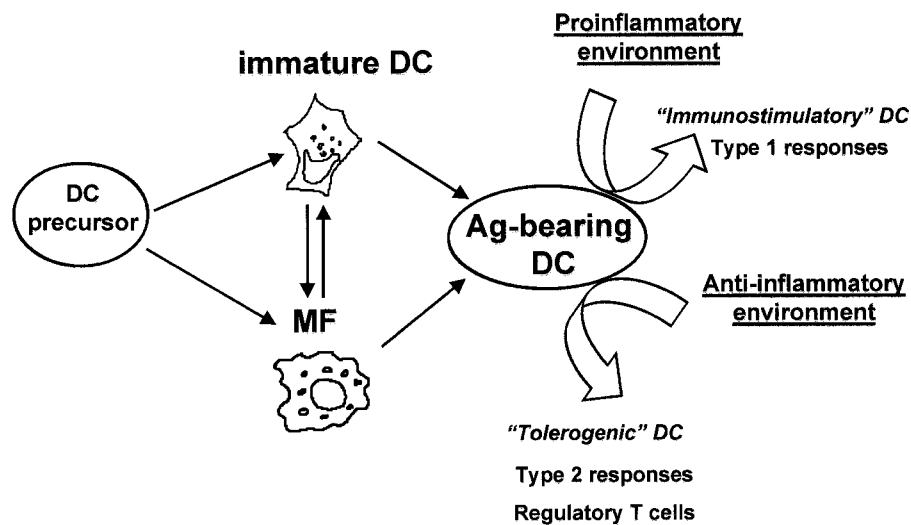
as discussed earlier. In some cases, T cells cluster around the mature DCs in peritumoral areas, thus resembling the DC/T cell clusters of secondary lymphoid organs, which are characteristic of ongoing immune reactions (75).

The unique ability of DCs to induce and sustain primary immune responses makes them optimal candidates for vaccination protocols in cancer (17, 251, 259). DCs loaded with appropriate TAAs can induce protective/rejection-immune responses in animal models (182, 260–266), and promising preliminary data are reported in humans (267–270). Several systems have been used to deliver TAA to DCs, including (a) defined peptides of known sequences, (b) undefined acid-eluted peptides from autologous tumors, (c) whole tumor lysates, (d) retroviral and adenoviral vectors, (e) tumor cell-derived RNA, (f) fusion of DCs with tumor cells, and (g) exosomes derived from DCs pulsed with tumor peptides (subcellular structures containing high levels of MHC molecules and peptides) (9, 251, 252). However, DC-mediated induction of immunity represents a challenge, and several parameters need to be considered to ensure the optimal outcome of DC-based vaccination protocols including (a) the source of TAA, (b) the methods for TAA preparation and loading, and (c) the diversity of DC subsets. These aspects are discussed elsewhere in this same volume (L Fong & E Engelman, ARI 18:245–73).

## DENDRITIC CELLS AND PATHOGENS

DCs have evolved to identify danger represented either as tissue damage or as a microbial invasion. Microbial products such as LPS or CpG DNA may activate innate immune mechanisms, including DCs, leading to responses beneficial to the host. However, pathogens have devised multiple strategies to evade immune responses by altering each step in the response, including inhibition of APC maturation and function (271), interference with MHC class I and class II processing/presentation pathways (viruses and bacteria), and hyperactivation of T cells (bacterial superantigens) to name a few. Recent studies are uncovering how pathogens can escape Ag-presenting functions of macrophages and DCs.

Bacterial LPS is a major molecule recognized by the innate immune system (6, 7). Ligation of membrane CD14 by complexes of LPS and soluble LPS-binding protein leads to proinflammatory signals including TNF and IL-1 secretion, which, when released at the site of tissue damage, increase the turnover of local APCs as well as recruitment of precursor cells (272). Toll-like receptor-4 (TLR4) transduces signal in LPS responses leading to NF- $\kappa$ B activation (5), and TLR4-deficient mice are hyporesponsive to LPS. TLRs contain a region in the intracellular domain that is homologous to part of the IL-1R and that is involved in the activation of IL-1R-associated kinase (273), leading to activation of several down-stream effectors. Furthermore, TLR2 but not TLR4 mediates responses elicited by components of gram-positive bacteria, such as peptidoglycan and lipoteichoic acid (274, 275). Although human monocytes transcribe Toll RNA and



**Figure 8** The plasticity of dendritic cells (DCs). According to microenvironmental instructions (e.g. cytokines), DC functions can be altered. For instance DCs can become macrophages (MF) with higher phagocytic functions in response to macrophage-colony-stimulating factor (M-CSF). Furthermore, interleukin (IL)-12 secreting DCs that induce type 1 T cell differentiation can switch, in response to IL-10 and prostaglandin E2, to DCs inducing T cell anergy or T cell differentiation into type 2 cells or regulatory T cells.

human macrophages release IL-12 on Toll-2 signaling, little is known regarding expression or function of Toll-like receptors on DC subsets (5).

After *in vitro* or *in vivo* exposure to LPS or other bacterial products, DCs undergo activation and maturation (91). *In vitro*, bacteria-induced DC maturation involves two signaling pathways: (a) ERK kinase, allowing for DC survival, and (b) NF- $\kappa$ B, allowing for DC maturation characterized by increased expression of costimulatory and MHC-class II molecules, release of chemokines, and migration. This coordinated process leads to high T cell-stimulatory capacity as well as IL-12 release (91), all of which result in the induction of protective immune responses.

DCs also initiate immune responses against parasites such as *Leishmania*. Immature DCs can phagocytose the organism, and LCs infected by *Leishmania* are present in the dermal infiltrate of skin lesions (92). *Leishmania*-infected LCs can migrate into the draining lymph nodes where they mature and activate *Leishmania*-specific T cells. Another parasite, *Toxoplasma gondii*, can induce the redistribution of DCs to T cell areas and activate the secretion of IL-12 by DCs but not by macrophages (26). Parasites can also subvert DC function to promote their own survival. A good example comes from the malaria parasite *Plasmodium*



*falciparum*, where *P. falciparum*-infected erythrocytes adhere to DCs and inhibit their maturation and capacity to stimulate T cells (276).

DCs are implicated in the pathogenesis/response to a variety of viruses, such as cytomegalovirus (CMV), human immunodeficiency virus (HIV), measles, herpes viruses, influenza virus, and most recently respiratory syncytial virus. DCs may be affected by viruses in several ways, including the following: (a) Because of their distribution throughout the body surfaces, DCs provide a means for viruses to access other cells; (b) persistent viruses may be sequestered within the DCs and may subvert DC function and thus escape immune surveillance, for instance CMV or HIV (277–279); (c) DCs may be susceptible to cytopathic effects of viruses, as shown in measles and HIV (280–282); and (d) viral double-stranded DNA can induce DC maturation and resistance to cytopathic effects of viruses, as shown recently in influenza. The acquisition of viral Ags by DCs may happen via (a) capture of virus-infected apoptotic cells, as for influenza (88); (b) expression of receptors as for HIV, in which DCs express both CD4, the receptor for HIV, and chemokine receptors that act as coreceptors for HIV (283); and (c) internalization of nonclathrin-coated caveolae as in respiratory syncytial virus infection (284). For the last two mechanisms, it remains to be determined whether the pathways of viral entry permit the access of viral Ags to DC processing/presentation machinery.

DCs contribute to the development of both nonclonal and Ag-specific antiviral responses. Interactions of blood CD11c<sup>+</sup> DC precursors with viruses leads to IFN- $\alpha$  release, initiating the cascade of antiviral response (55, 58, 66) mediated primarily by direct and indirect (IFN $\gamma$ ) cytotoxicity of NK cells, NK T cells, and macrophages (Figure 2). Development of subsequent clonal immunity may differ depending on the virus. For instance, DCs infected with wild-type measles virus, as well as the vaccine strains, eventually undergo apoptosis and are unable to stimulate proliferation of alloreactive T cells. Although this can explain the profound immunosuppression caused by measles, it becomes unclear how immunity against measles is ever established. One possibility is that noninfected DCs may capture measles virus-induced apoptotic bodies, as occurs with influenza virus, and subsequently initiate CTL responses. Alternatively, measles virus may differentially affect the various DC subsets or maturational stages, as evidenced by the fact that measles virus-infected immature DCs induce T cell death, whereas infected mature DCs do not (280–282).

Viruses can alter DC functions by interaction with inhibitory leukocyte Ig-like receptors (LIR) (285). Indeed, the UL18 glycoprotein of CMV, homologous to MHC class I, is a decoy ligand for LIR1 expressed on myelomonocytic cells including monocytes and likely DCs (285). Several functional consequences are possible, including both inhibition of differentiation and cytokine release and downregulation of antiviral response or subversion of the negative regulatory function of LIR, ultimately allowing viral replication. The DC system is also involved during HIV infection (286, 287). Thus, DCs can act as (a) transporters of the HIV, initially deposited on the mucosa, to activated T cells in secondary

lymphoid organs and (b) permissive sites for virus replication. Indeed, cocultures of DCs and T cells permit HIV replication that seems to occur within syncytia that are heterokaryons of DCs and T cells. Such HIV-expressing syncytia have been found in vivo at the surfaces of mucosal lymphoid tissues like tonsils and adenoids (280). HIV-induced cell fusion of DCs and memory T cells brings together at least two transcription factors, such as NF- $\kappa$ B and Sp1, the coexpression of which, in heterologous syncytia, permits virus transcription and chronic replication (288). Furthermore, patients with high viral loads have decreased proportions of DC precursors in the blood, which may contribute to immunodeficiency during HIV infection (289).

Much remains to be learned about the interactions of DCs and viruses and how persistent viruses like CMV or HIV subvert in vivo DC function and/or maturation. This is of particular importance because manipulation of the DC system could permit control of viral infections, for instance by increasing the activity of IFN $\alpha$ -producing CD11c<sup>+</sup> DCs.

## CONCLUDING REMARKS

Dendritic cells induce, sustain, and regulate immune responses. Several key features of dendritic cells can be highlighted: (a) the existence of different DC subsets that share biological functions, yet display unique ones such as polarization of T cell responses towards type 1 or type 2 or regulation of B cell responses; (b) the functional specialization of DCs in relation to their differentiation/maturation stages, including (i) ability to secrete large amounts of pro-inflammatory and/or anti-viral cytokines at their precursor stage, (ii) high Ag capture capacity at their immature stage, and (iii) ability to activate and modulate T cell responses at their mature stage; (c) the plasticity of DC functions, which is determined by the micro-environment (e.g. cytokines) and may manifest as (i) the final differentiation into either DC (enhanced Ag presentation) or macrophage (enhanced Ag degradation), (ii) the induction of immunity or tolerance, and (iii) the polarization of T cell responses towards type 1 or type 2.

The next few years will undoubtedly increase our understanding of the pathophysiology of DCs. We expect the genomic studies to yield molecules that will permit better definition of DC heterogeneity and explain the unique biological functions of DCs. The current clinical trials with ex vivo-generated DCs will yield precious information regarding their potential as vectors for immunotherapy. Ultimately we predict that DCs will be targeted in vivo by "intelligent missiles," man-made viruses composed of a lipid envelope expressing specific ligands that can bind to either all DCs or to a specific subset. The missile may be loaded with (a) DC modulators (activators or inhibitors) to induce or suppress a given immune response or (b) Ags together with DC modulators for vaccination. This is enough to keep us busy for a while.

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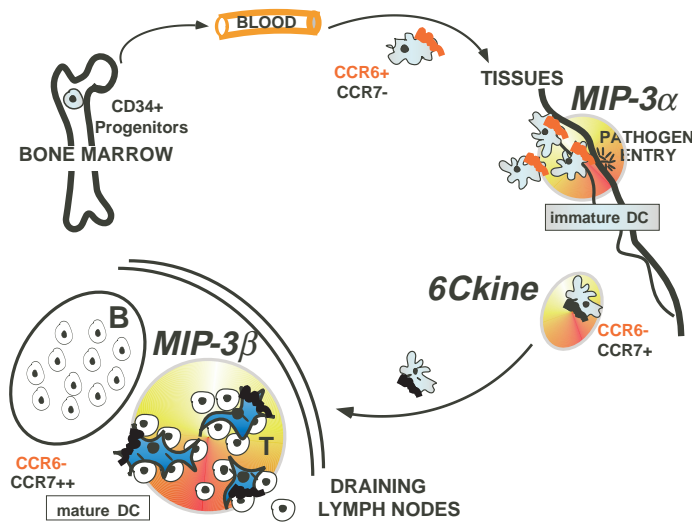
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**Figure 3** The critical role of chemokines in a dendritic-cell odyssey. Precursor and immature Langerhans cells that display CCR6 are attracted by the epithelium that expresses the specific ligand MIP-3α. Upon antigen capture and activation, Langerhans cells detach from keratinocytes by down-regulating E-cadherin, and they traverse the basal membrane by secreting proteases such as collagenase. Meanwhile CCR6 is replaced by CCR7, whose ligands are (a) 6Ckine, which is expressed on lymphatic vessel walls, and (b) MIP-3β, which is expressed in the T cell areas of lymphoid organs. This may guide the maturing DCs to the T cell areas where they will start to produce chemokines that attract lymphocytes.