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Human Dendritic Cell Subsets for Vaccination

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Protective immunity results from the interplay of antigen (Ag)-nonspecific innate immunity and Ag-specific adaptive immunity. The cells and molecules of the innate system employ nonclonal recognition pathways such as lectins and TLRs. B and T lymphocytes of the adaptive immune system employ clonal receptors recognizing Ag or peptides in a highly specific manner. An essential link between innate and adaptive immunity is provided by dendritic cells (DCs). As a component of the innate immunc system, DC organize and transfer information from the outside world to the cells of the adaptive immune system. DC can induce such contrasting states as active immune responsiveness or immunological tolerance. Recent years have brought a wealth of information regarding DC biology and pathophysiology that shows the complexity of this cell system. Thus, presentation of antigen by immature (non-activated) DCs leads to tolerance, whereas mature, antigen-loaded DCs are geared towards the launching of antigen-specific immunity. Furthermore, DCs are composed of multiple subsets with distinct functions at the interface of the innate and adaptive immunity. Our increased understanding of DC pathophysiologywill permit their rational manipulation for therapy such as vaccination to improve immunity.

KEY WORDS: Dendritic cell; pathogens; vaccination; T cell immunity; subsets.

INTRODUCTION

The immune system evolved to protect us from microorganisms. The antigen (Ag)-nonspecific innate immunity and Ag-specific adaptive immunity act in concert to eradicate pathogens, through cells, such as macrophages, granulocytes, dendritic cells (DCs), and lymphocytes, and through effector proteins such as cytokines, antimicrobial peptides, complement, and antibodies (1–3). Lymphocytes (T cells, B cells, NK, and NK T cells) and their products are under the control of DCs (4–7). DCs sit in peripheral tissues where they are posed to capture antigens (Fig. 1). Antigen-loaded tissular DCs migrate through the afferent lymphatics into the draining lymph nodes. There, they present processed protein and lipid Ags to T cells via both classical (MHC class I and class II) and non-classical (CD1 family) antigen presenting molecules (6). Immature (non-activated) DCs present self-antigens to T cells (8–10), which in the absence of appropriate costimulation leads to tolerance. Mature, antigen-loaded DCs are geared toward the launching of antigen-specific immunity (11) with T cell proliferation and differentiation into helper and effector cells with unique function and cytokine profiles. However, DCs can induce immune tolerance partly through T cell deletion and partly through activation of regulatory T cells. DCs are composed of multiple subsets with distinct functions at the interface of the innate and adaptive immunity. How this complex balance is maintained in health and broken in disease; and how it is regulated through distinct DC subsets and their functional plasticity is now starting to be understood. We will focus on recent progresses in our knowledge of the physiology of DCs. In particular, on the identification of distinct DC subsets that induce distinct types of immune response (6, 12, 13) and how this new understanding of ways by which DCs regulate immunity might impact the design of novel vaccines.

BIOLOGY OF DENDRITIC CELLS

DCs Capture and Present Antigens

Immature DCs are remarkably efficient in Ag capture whereas mature DCs are remarkably efficient in antigen presentation. DCs utilize several pathways to capture antigen including (1) macropinocytosis; (2) receptor-mediated endocytosis via C-type lectins (for example

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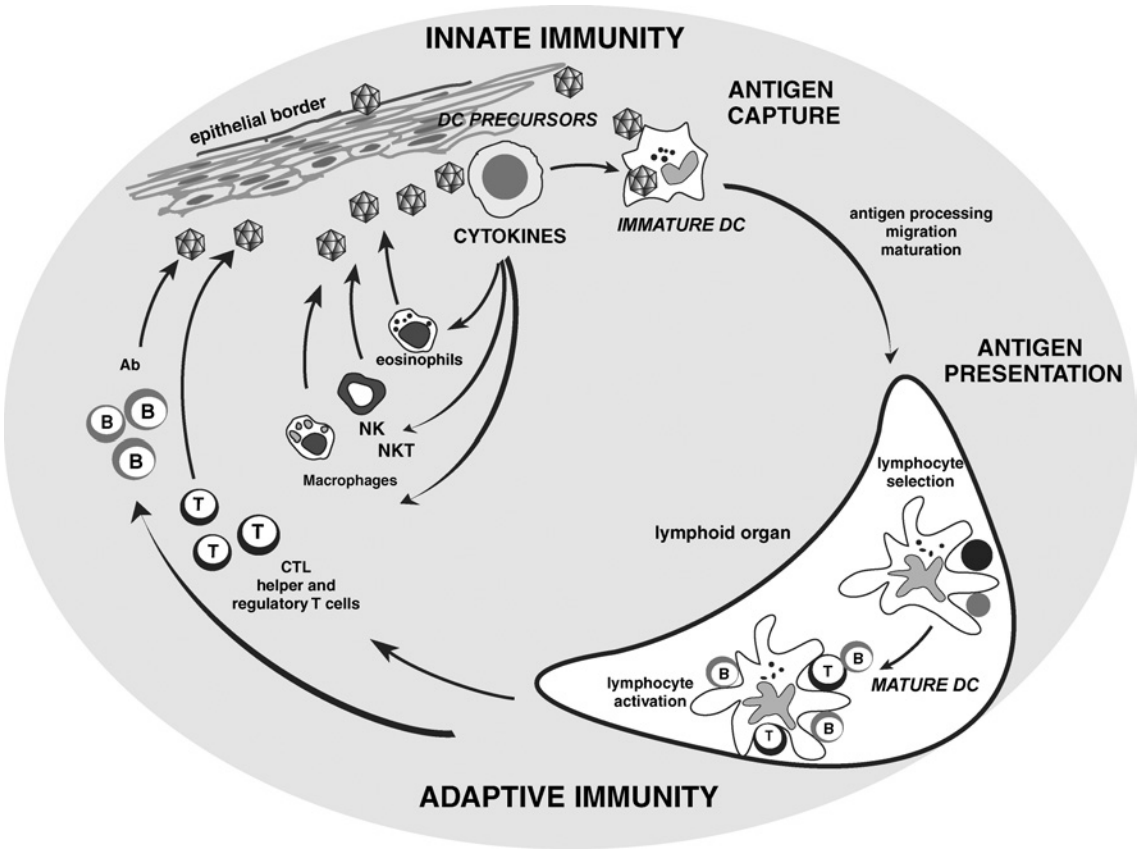

Fig. 1. The life cycle of dendritic cells. Circulating precursor DC enter tissues as immature DC. They can encounter pathogens (e.g., viruses) directly, which induce secretion of cytokines (e.g., IFN-α); or indirectly through pathogen effect on stromal cells. Cytokines secreted by DCs in turn activate effector cells of innate immunity such as eosinophils, macrophages, and NK cells. Microbe activation triggers DCs migration toward secondary lymphoid organs and simultaneous maturation. Mature DCs that enter lymphoid organs display pMHC complexes, which allow selection of rare circulating antigen-specific T lymphocytes. These activated T cells help DCs for their terminal maturation, which allow lymphocyte expansion and differentiation. Activated T lymphocytes traverse inflamed epithelia and reach the injured tissue, where they eliminate microbe and/or microbe-infected cells. B cells, activated by DCs and T cells, migrate into various areas where they mature into plasma cells that produce antibodies that neutralize the initial pathogen.

mannose receptor, DEC-205, DC-SIGN) (14–21) or Fcγ receptors type I (CD64) and type II (CD32) (uptake of immune complexes or opsonized particles) (22); (3) phagocytosis of apoptotic and necrotic cells (8, 9, 23), viruses, bacteria including mycobacteria (24, 25), as well as intracellular parasites such as *Leishmania* major, and (4) internalization of heat shock proteins, hsp70 or gp96-peptide complexes, through multiple receptors including LOX-1 (26) and TLR2/4 (27). Captured antigens are processed in distinct intracellular compartments and loaded onto DCs antigen presenting molecules (reviewed in (28)). Protein antigens are presented by classical MHC class I and class II molecules while lipid antigens are presented through non-classical CD1 antigen presenting molecules (6).

Presentation via MHC Class II and Class I. Captured antigens are presented by MHC class II molecules (29) which upon DC maturation are transported from lysosomal compartments to the cell membrane (30, 31). In fact, this translocation of peptide-MHC (pMHC) class II complexes from intracellular compartments to cell membrane represents a hallmark of DC maturation. pMHC complexes are very stable on the cell membrane of mature DCs thereby facilitating TCR recognition. Furthermore, as opposed to macrophages that favor antigen degradation, DCs show lower levels of lysosomal proteases thereby permitting low rate of antigen degradation (32). This in turn permits antigen retention in lymphoid organs *in vivo* for extended periods that might favor antigen presentation (32). Thus, the prolonged availability of antigen for generation of

pMHC complexes and prolonged presentation of such complexes on cell surface might both explain a unique efficiency of DCs in triggering naive T cell differentiation.

MHC class II molecules are under the control of a transcriptional coactivator, MHC class II transactivator (CIITA) (33). The expression of CIITA is regulated by three independent promoters the activity of which quantitatively determines MHC class II expression (34). Distinct subsets of antigen presenting cells utilize different promoters, i.e., plasmacytoid DCs (pDCs) and B cells rely on promoter pIII whereas myeloid DCs and macrophages use pI (35). These differences may have fundamental impact on the antigen presentation on MHC class II by these cell types and on ensuing immune responses.

MHC class I molecules represent another antigen presentation pathway exploited by DCs (28). This involves the classical presentation of endogenous peptides, originating from cellular and viral proteins, as well as the presentation of exogenous antigens via cross-priming/presentation. In fact, cross-priming/presentation might be the main pathway through which immunity to tumors and microbes that do not infect DCs directly is generated (8, 36, 37). This pathway is being now exploited for loading antigens on DC vaccines as discussed later. It remains to be defined how DCs are able to put exogenous antigens for presentation on MHC class I. Cross-priming might actually be based on the transfer of proteasome substrates rather than peptides (38). Furthermore, the loading compartment remains as yet an unresolved issue, i.e., whether it is the endoplasmic reticulum (ER) or a mixed phagosome-ER compartment (39). It is not clear whether loading MHC class I with exogenous 9–10 AA peptides as used for vaccination in cancer (see later) actually happens *in vivo*.

Presentation via CD1 Family of Non-Classical MHC Molecules. CD1 proteins present lipid antigens to effector T cells (40, 41). In the human, but not in the mouse, this family consists of four members CD1a–c (group 1 molecules) and CD1d (group 2 molecules), with a distinct expression patterns (40, 41). Different CD1 molecules display distinct intracellular trafficking patterns likely resulting in antigen delivery into distinct compartments (40, 41). Differential expression of CD1 molecules and its impact on T cell immunity can be illustrated by CD1a and CD1d. CD1a expression *in vivo* is restricted to Langerhans cells (LCs) and thymocytes. LCs have been shown to use CD1a and Langerin (a unique lectin expressed by LCs) to present nonpeptide antigens of *Mycobacterium leprae* to T cell clones derived from a leprosy patient (42). CD1a-restricted T cells indicated that lipopeptide antigen presentation by CD1a involves the anchoring of antigens in the hydrophobic binding groove, resulting in exposure of the peptide moiety for TCR contact (43). CD1d is unique in that it is involved in the antigen presentation to natural killer (NK)T cells, a unique subset of T cells expressing a limited TCR repertoire mostly composed of Vα24Vβ11 (44). These innate-like T cells contribute to immune response to infection and malignancy. Recent studies identified lysosomal glycosphingolipid, isoglobotrihexosylceramide (iGb3) (45), as endogenous and bacterial glycosylceramides (46) as exogenous antigens for presentation by CD1d to NKT cells. Interestingly, CD1d ligation on monocytes triggers translocation of NFκB and IL-12 secretion (47) thus providing a possible mechanism through which NKT cells modulate antigen presenting cells and immune responses (48). The next challenge will be to understand which pathways of antigen presentation are being preferentially utilized by distinct DC subsets, and the consequences of such differential presentation on the outcomes of immune responses.

DCs Migrate and Orchestrate Migration of Other Cells

During their life span, DC migrate from the bone marrow through blood to peripheral tissues and to lymphoid tissues. Both DCs migration and their capacity to orchestrate the migration of immune effectors are fundamental for the launching and the coordination of immune responses.

Migratory Pathways. DC migration to the periphery and from the periphery to lymphoid tissue represents two separate events that are regulated by distinct sets of molecules. Immature/non-activated DCs patrolling via blood are attracted to tissues for example through MIP3-α (via CCR6) or MCP chemokines (via CCR2) as demonstrated for LCs *in vitro* (49) and *in vivo* (50), respectively. Interestingly, monocyte derived-DC respond to MIP1-α/β (via CCR1 and CCR5) but not to MIP3-α (51, 52), suggesting that distinct DC subsets might utilize distinct migratory pathways. Upon pathogen entry respective ligands are secreted by epithelial cells thus providing an impetus for enhanced DC influx. Indeed, Holt *et al*. found that DCs are the first to arrive at the site of pathogen entry, preceding even neutrophils (53–55). Furthermore, we have found that both mDCs and pDCs are attracted *in vivo* to the respiratory tract in children with acute viral infections triggered by Influenza virus or RSV (56). Much less is known about how DCs enter and traffic through the lymphatic vessels. The chemokine receptor CC-chemokine receptor 7 (CCR7) appear to be fundamental in this process (57). Thus, distinct maturation/activation signals, for example prostaglandin PGE2 (58, 59), that induce the preferential expression of CCR7 by DCs, might increase the capacity

of the DCs to respond to appropriate ligands such as CCL19 and CCL21 expressed in lymphatic vessels and secondary lymphoid organs (60).

An important question is whether all DCs are equal in their migration routes and mechanisms. In fact, human tonsils contain DCs with different phenotypic characteristics at strategically different location within the lymphoid tissue (61, 62). There, DCs within germinal centers are phenotypically different from DCs located within and around HEVs that have been demonstrated to be actually pDCs (62, 63). This suggests that distinct DC subsets might approach secondary lymphoid organs through distinct routes. Indeed, upon bacterial triggering mDC precursors migrate to peripheral tissues and subsequently to draining lymph nodes, while pDC precursors directly enter the lymph nodes in a CXCL9 and E-selectin dependent manner (64). Differential migration of cutaneous DC subsets, i.e., LCs and dermal DCs has been demonstrated recently with the use of vital imaging in knockin mice expressing enhanced green fluorescent protein (EGFP) under the control of the Langerin (CD207) gene (65). Thus, after skin immunization, dermal DCs arrived in lymph nodes first and colonized areas distinct from slower migrating LCs (65). Theses results might in fact contribute to our understanding of how the immune response develops upon cutaneous immunization, a classical route of vaccination. The impact of DC migration on T cell immunity is discussed later.

Molecules Regulating DC Migration. These belong to three major categories that include chemokines (66), adhesion molecules (67) and more recently products of lipid metabolism (68, 69). The classical view as mentioned above is that of chemokines MIP3-α (CCR6 ligand) and MIP-3β (CCR7 ligand) representing the two major forces regulating migration of myeloid DCs to periphery and to lymphatics, respectively (70). An emerging view however is that of a coordinated action of several chemokines and adhesion molecules as perhaps best illustrated by plasmacytoid DCs (pDCs). There, the entry of pDC to either peripheral tissue or lymphoid organ is a net result of (1) their expression of adhesion molecules such as the skin homing molecule cutaneous lymphocyte antigen (CLA) or lymph node homing molecule L-selectin and (2) the cooperation between inducible CXCR3 ligands and constitutive SDF-1/CXCL12 expression to which pDCs can migrate (71). The response of DCs to chemokines might be further regulated by products of lipid metabolism (69). Thus, triggering DCs with leukotriene C(4) enhances their chemotaxis to CCL19 (CCR7 ligand) (72). Accumulation of lipids is in turn controlled by multidrug resistance proteins (72). This mechanism might be particularly important for the regulation of DC entry and pass through lymphatic vessels (69). Furthermore, it might be particularly relevant in the inflammatory response where lipid mediators play a central role.

DCs Orchestrate the Migration of Immune Effectors. DCs attract immune effectors through chemokines (73–79) and regulate their maturation and function through cell–cell contact, and/or soluble factors (4–7, 80). The analysis of chemokine secretion by blood DC subsets exposed to influenza virus permitted us to determine a sequential chemokine secretion program common to the two subsets, i.e., pDCs and mDCs. This program, illustrated in Fig. 2, might actually explain how the DCs coordinate the launching of immune response as they mature and migrate toward lymphoid organs. Thus, the first chemokines to be produced are those attracting innate effectors and cytotoxic cells, which might permit to limit the spread of infection. The next wave involves the production of chemokines able to attract memory T cells. Finally, as mature DCs land in the secondary lymphoid organs they secrete chemokines that attract B cells, allowing for antibody production, and naïve T cells, allowing their priming. Attraction of T cells with regulatory/suppressor function might finally permit the termination of immune response. Accordingly, CXCL13 have been shown to attract B cells as well as CXCR5+CD4 memory T cells able to enhance IgG and IgA production (81). Furthermore, CXCL13 producing cells are located uniquely in the follicles and germinal centers (82). Thus, Influenza virus activated DCs that migrate to B cell areas of peripheral lymphoid organs (83) could launch humoral immunity via CXCL13 whereas those that migrate to T cell zone could launch T cell immunity via CCL19 and CCL22 (84, 85).

DC Maturation

DC migration is intimately linked with their maturation and, consequently, with their impact on T cell immunity.

Maturation Signals. DCs can receive maturation signals through (1) cells including T cell derived CD40 ligand (86); as well as signals from NK, NKT cells and γ/δ T cells (reviewed in (48)); (2) cell products such as proinflammatory cytokines including IL-1β, TNF, IL-6, and PGE2 (87), or an apparently more potent combination of IL-1β and TNF with type I (IFN-α) and II (IFN-γ) interferons (88); and (3) DC surface molecules involved in pathogen recognition including Toll receptors (TLRs) and C type lectins (reviewed in (89)). Most likely at any given time point the DCs will be exposed to a combination of these signals which will influence the net result of T cell activation as discussed later. Interestingly, TLRs are differentially expressed by distinct DC subsets. For example, TLR9 (a receptor for demethylated DNA) is expressed

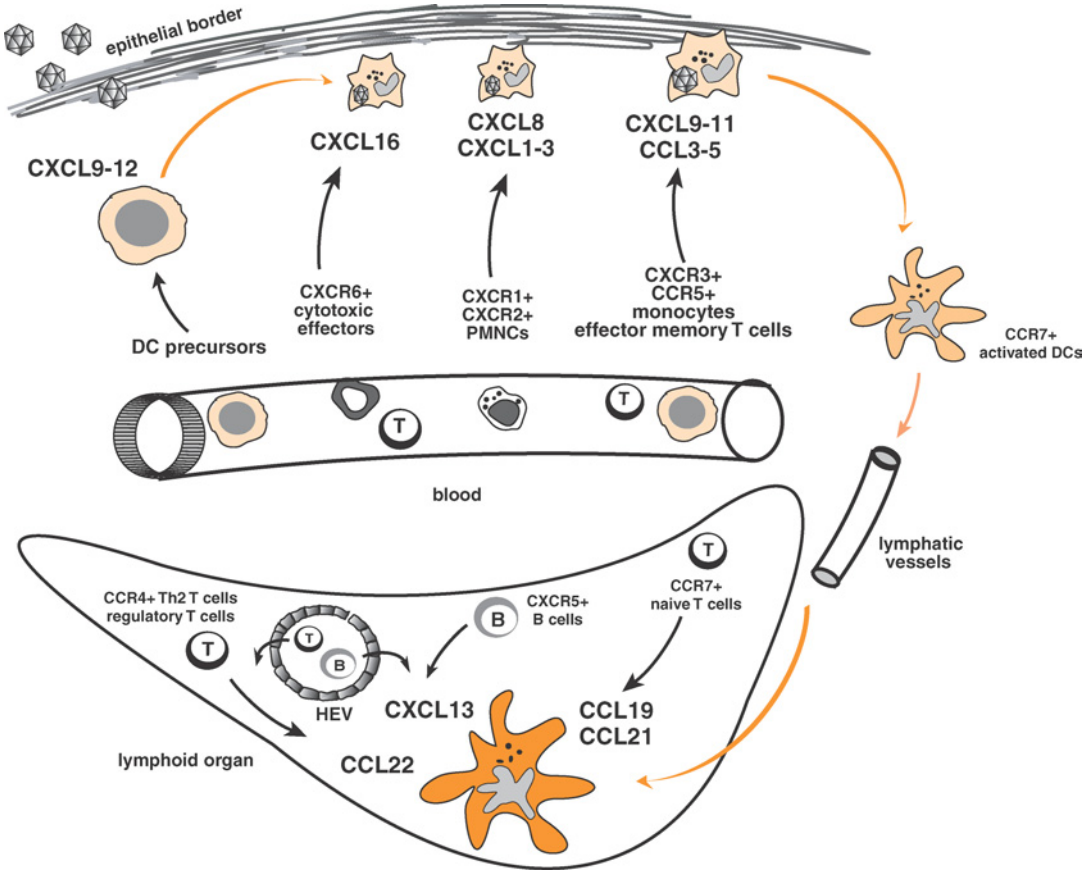

Fig. 2. DCs orchestrate the migration of immune effectors. Blood DCs expressing at the steady state CXCR4 and CXCR3 can migrate through virally infected tissues, expressing CXCL12, CXCL9, CXCL10, and CXCL11. There, they can roll on inflamed endothelial cells; adhere to them and penetrate the tissues. Upon encountering the virus in the tissues, they start to release at the first step CXCL16, CXCL1, CXCL2, CXCL3, CXCL7, and CXCL8. These chemokines attract Th1 effector cells expressing CXCR6 and neutrophils expressing CXCR2. Later, activated DCs secrete CCL2, CCL3, CCL4, CCL5, and CCL8, which essentially attract CCR5 expressing memory T lymphocytes and monocytes. Upon maturation, DCs up-regulate CCR7 and down-regulate CXCR4, allowing their migration, toward CCL21, through lymphatics into secondary lymphoid organs. In the lymphoid organs, mature DCs secrete CCL19 and CXCL13, which respectively attract CCR7 expressing naïve T cells and CXCR5 expressing naïve B cells. They also secrete CCL22, attracting CCR4 expressing Th2 and CD4+CD25+ regulatory cells.

only by pDCs whereas mDCs preferentially express TLR 2 and 4 (receptors for bacterial products such as peptidoglycan and lipopolysaccharide, respectively) (90). Similarly, and as discussed later, distinct DC subsets express unique lectins (20), which can display immunostimulatory (ITAM) or inhibitory (ITIM) motifs. Such differential expression may confer distinct maturation signals yielding distinct type of immune responses (91). Beside a direct triggering of TLRs and C type lectins on DCs, an important concept for understanding of pathogen/DC interactions is the indirect effect. There, TLR-mediated signaling of stromal cells will trigger expression of a specific of chemokines/cytokines and adhesion molecules, which in turn will modulate DC maturation (92).

Maturation Phenotype. The DC maturation is a continuous process that is associated with several coordinated events such as (1) loss of endocytic/phagocytic receptors; (2) upregulation of costimulatory molecules CD40, CD80, CD86, and several members of TNF/TNF receptor family including CD70 (ligand for CD27), 4-1BB-L, and OX40-L, all of which can have costimulatory effects on T cells (93).; (3) change in morphology, that include a loss of adhesive structures, cytoskeleton desorganization, and the acquisition of high cellular motility (94); (4) shift in lysosomal compartments with down-regulation of CD68 and up-regulation of DC-LAMP (95); (5) change in class II MHC compartments as discussed above; and (6) secretion of cytokines including IL-12 and IL-23,

which are important for the type 1 polarization of T cell immunity.

This basic process of DC maturation can be modulated by pathogens via interaction with TLRs expressed on DCs. For example, TLR ligands together with a T cell-like signal delivered through CD40, may enhance DC function (96). Indeed, TLR-mediated signals are involved in the control of $CD4^+$ T-cell activation (97) and, for example, DCs loaded with a heart-specific self-peptide induce $CD4^+$ T-cell-mediated myocarditis in nontransgenic mice if activated through both CD40 and TLRs (98). Pathogens may also contain several TLR agonists that could engage several TLRs on the same DCs or on two distinct DC subsets (3). Thus, for both human and mouse DCs, TLR3 and TLR4 acted in synergy with TLR7, TLR8, and TLR9 leading to increased production of IL-12 and IL-23 (99). This was accompanied by an increase in the ratio of Delta-4/Jagged-1 that are dictating the type of T cell immunity elicited by DCs (100). As expected this led to sustained T helper type 1-polarizing capacity of exposed DCs (99). While we will discuss the type 1/type 2 modulation in the next chapter, these results suggest that TLR signaling might polarize DC maturation toward Th1 or Th2 inducing cells by modulating Notch ligands on DCs.

Functional DC maturation can also be modulated by C type lectins. Thus, Dectin-1, a yeast binding C type lectin synergizes with TLR2 to induce TNF alpha and IL-12 (101). Yet, Dectin-1 can also promote synthesis of IL-2 and IL-10 through recruitment of Syk kinase. Accordingly, syk−/− DCs do not make IL-10 or IL-2 upon yeast stimulation but produce IL-12, indicating that the Dectin-1/Syk and Dectin-1/TLR2 pathways can operate independently (101). These results bring an important demonstration that pathogens utilize several surface molecules to modulate DC function.

Dendritic Cells Determine the Type of T Cell Response

DCs control lymphocyte priming and the type of induced T cell immunity. This determination is intimately linked with several aspects of DC biology including DC migration, and maturation and finally distinct DC subsets as discussed later.

DCs Migration. The physical location of DCs at the time of antigen capture and at the time of antigen presentation is important for ensuing T cell immunity. For example, upon subcutaneous immunization with fluorescent antigen two waves of DC-mediated antigen presentation occur (102). The first one is mediated by DCs that acquired the antigen directly in the draining lymph nodes (102). DCs migrating from the actual antigen injection site and presenting the captured antigen arrived several hours later in the second wave (102). The first wave of DCs triggered T cell activation and proliferation while the second wave was necessary for development of delayed-type hypersensitivity (102). Furthermore, after skin immunization, dermal DCs and LCs (65) colonized distinct locations within the paracortical, T cell-rich zone (65). Thus, dermal DCs colonized the outer paracortex, at the junction with B cell follicles, whereas LCs migrated into the inner paracortex (65).

Tissue origin of DCs determine the homing of elicited T cells (103, 104). Thus, whereas DCs from Peyer's patches, peripheral lymph nodes, and spleen induced equivalent activation markers and effector activity in $CD8^+$T cells, only Peyer's patch DCs induced $CD8^+$T cells with the ability to home to the small intestine (103, 104). Similarly, when *ex vivo* generated DCs were injected into mice bearing melanoma, both intravenous and subcutaneous injection induced specific memory T cells in spleen and permitted control of lung metastasis. However, only subcutaneous immunization permitted subsequent protection against subcutaneously growing tumors (104). Thus, DCs that have migrated to different tissues can prime T cells with different homing capacities. Finally, inflammation will enhance DC migration as demonstrated in mice by conditioning the site of *ex vivo* generated DC injection with TNF. This in turn significantly increased DC migration to the draining lymph nodes and the magnitude of the $CD4^+$ T-cell response (105).

DCs migration within lymphatics and to specific areas within lymphoid organs represents another important parameter for T cell immunity. We have already discussed the strategic location of distinct DCs with specific areas of secondary lymphoid organs as analyzed on tissue sections. Furthermore, it appears that DCs recirculate within lymphoid organs possibly to deliver help to each other as shown recently for pDCs delivering help for mDCs to prime Herpes Simplex virus specific CTLs (106). This important aspect of DC biology can be now appreciated *in vivo* thanks to development of intravital imaging technologies (107). This will allow to dissect *in vivo* the DC and lymphocytes interaction in steady state (108) and upon launching of protective immunity. For example, it appears that both tolerance and immunity are proceeded by stable and lasting several hours interaction of DCs with T cells (108).

DC Maturation and the Outcome of Interaction with T cells. The current paradigm is that immature DC are tolerogenic wheras mature DCs are immunostimulatory (109). This has been formally demonstrated *in vivo* by the groups of Nussenzweig and Steinman who have elegantly shown that fusion proteins targeted to immature DCs lead to the

induction of antigen-specific tolerance (110). By contrast, concomitant activation of the DCs with CD40-specific antibody results in a potent immune response as DCs are induced to express a large number of costimulatory molecules (111). However, mature LPS-activated DCs efficiently expand $CD25^+CD4^+$ regulatory T cells (112). This raises a question whether each type of DC maturation will lead to an immunostimulatory DCs as discussed later.

Immature steady-state DCs have been considered important in peripheral tolerance possibly through presenting tissue antigens without appropriate costimulation. Recent studies call to revisit this paradigm because LCs reaching lymph nodes under steady-state or inflammatory conditions were found to express similar levels of MHC class II, CD40, and CD86 (65). Thus, the determination of tolerance or priming might be related to the threshold of activation (113) and/or action of a unique set of inhibitory molecules, such as signaling through CD80/CD86 and CTLA-4 or PDL1/2 and PD-1 or Immunoglobulin-like transcript 3 (ILT3) and ILT4 (114–116) as discussed later.

DC Maturation and the Type of Induced T Cell Immunity. TLR ligands are among the DC activation/maturation signals that have a profound effect on the type of elicited immunity. We have already discussed above the synergistic effect of multi-TLR signaling on Th1 response. Another response scenario in which distinct TLR ligands induce distinct polarization of DCs can be illustrated by TLR2 and TLR4 ligands. Thus, minor structural differences in TLR ligands such as LPS may lead to the engagement of different TLRs, as illustrated by *E. coli* LPS, which induces a Th1 response via IL-12 secretion, whereas *Porphyromonas gingivalis* LPS, which triggers TLR2, induces a Th2 response (117). Furthermore, *E. coli* LPS (TLR4 ligand), activates DCs to produce abundant IL-12(p70), but little IL-10, thereby promoting type 1 responses. In contrast, Pam-3-cys (TLR2 ligand) elicits less IL-12(p70), but abundant IL-10, thereby promoting type 2 responses. This regulation appears to be mediated via extracellular signal-regulated kinase and c-fos signalling in DCs exposed to TLR2 ligand (118). Thus, TLR signalling might polarize DCs toward Th1 or Th2 inducing cells by modulating signalling pathways in DCs (101).

DC Maturation and Regulatory/Suppressor T Cells. Two broad subsets of $CD4^+$ T cells with regulatory function have been characterized (119–121), both of which can be activated/expanded by DCs at distinct maturation stages.

Naturally occurring $CD4^+CD25^+$T cells are produced in the thymus and mediate their suppressive effects in a cell contact-dependent, antigen-independent manner, without the requirement of IL-10 or TGF-β(122–125). These cells are naturally "anergic" and require stimulation via their TCR for optimal suppressive function. Mature DCs allow their expansion which is partially dependent on the production of IL-2 by the T cell and B7 co-stimulation by the DCs (112).

The induced T regulatory (T_R) cells: T_R derive from $CD4^+25^-$ T cells and mediate their effects through the production of suppressive cytokines such as IL-10 and TGF-β(126–128). Two types have been described: T_R1 cells that produce large amounts of IL-10 and low to moderate levels of TGF-β (126) and Th3 cells that produce preferentially TGF-β (129) and provide help for IgA production (130). Immature DCs induce the differentiation of naïve T cells into T_R cells (126, 131, 132). Injection of immature DCs pulsed with influenza-derived peptide has been shown in two healthy adults to lead to antigen-specific silencing of effector T-cell function (133). Murine pulmonary DCs induce the development of T_R in an ICOS-ICOS-L-dependent fashion which leads to the production of IL-10 by DCs (134). Furthermore, a population of "semi-mature" $CD45RB^{high}$ $CD11c^{low}$ murine DCs located within the spleen and lymph nodes has been described. These cells secrete IL-10 after activation with LPS or CpG oligonucleotides but do not upregulate MHC class II or co-stimulatory molecules under the same conditions. Most importantly, they are highly potent at inducing tolerance that is mediated through the differentiation of T_R cells *in vivo* (132, 134). The complexity of the lineage and/or the subpopulations of DCs that may be responsible for tolerance induction is further illustrated by the description of unconventional DCs (135) that display phenotypic and functional properties of both natural killer (NK) and dendritic cells (DC). These cells appear able to induce protection against virally induced type-1 diabetes in a mouse model.

DC Subsets and the Type of Induced T Cell Immunity. Finally, distinct DC subsets differentially modulate T cell immunity. A detailed discussion of DC subsets follows in the next chapter. Here we will briefly summarize the role of DC subsets in T cell polarization.

In mice, splenic $CD8\alpha^+$ DCs prime naïve $CD4^+$ T cells to make Th1 cytokines in a process involving IL-12, whereas splenic $CD8\alpha^-$ DCs prime naïve $CD4^+$ T cells to make Th2 cytokines (136, 137). Furthermore, different signals can induce different T-cell polarization by the same DCs, as shown by the induction of IL-12 production and Th1-cell polarization when DCs are activated with *E. coli* LPS, but no IL-12 production and Th2-cell polarization when DCs are exposed to LPS from *P. gingivalis* (117). In humans, CD40-ligand (CD40-L)-activated monocyte-derived DCs prime Th1 responses through an IL-12-dependent mechanism, whereas pDCs activated with IL-3 and CD40-L have been shown to

secrete negligible amounts of IL-12 and prime Th2 responses (138). Furthermore, IL-3 and CD40-L activated pDCs induce $CD8^+$T cells with regulatory/suppressor function (139, 140). Thus, both the type of DC subset and the activation signals to which DCs are exposed are important for T cell polarization.

Dendritic Cells are Composed of Subsets

In early 90s, culture systems were discovered that produced large amounts of mouse (141) and human DCs thereby accelerating their characterization (142–144). Classically, two main DC differentiation pathways are recognized (6, 7). A myeloid pathway generates Langerhans cells (LCs), which are found in stratified epithelia such as the skin, and interstitial (int)DCs, which are found in all other tissues (145). Another pathway generates plasmacytoid DCs (pDCs) (146), which secrete large amounts of IFN-α/β after viral infection (63, 147, 148).

DC Progenitors and Precursors. DC progenitors reside within $CD34^+$ hematopoietic progenitor cells (HPCs) (142). Both lymphoid and common myeloid progenitors yield, at the clonal level, mDCs as well as cells with pDCs phenotype and capacity to secrete large amounts of IFN-α (149). Interestingly, the progenitors of pDCs and mDCs can be found within $FLT3^+$ HPCs (150, 151). This is consistent with the well established role of FLT3 ligand (FLT3-L) in DCs differentiation/mobilization *in vivo* in both humans and mice (152–156). Accordingly, FLT3-L is essential in the generation of pDCs and myeloid DCs (mDCs) (157–159), and FLT3-L deficient mice show a considerable decrease in numbers of DCs in both peripheral and lymphoid tissues (152). Thus, FLT3-L appears as a major factor governing DC homeostasis in the steady state. Given the role of GM-CSF in DC generation (142, 160, 161), activation and survival (162), it is tempting to postulate that GM-CSF is actually a major factor governing DC homeostasis on infection. In this context, GM-CSF preferentially expands the myeloid DC subset *in vivo* (137). Furthermore, GM-CSF gene-targeted mice (GM−/−) show delayed clearance of *group B streptococcus* from the lungs as compared to wild-type mice (163).

Until recently monocytes and pDCs have been considered as major circulating DC precursor populations. However, recent studies demonstrated that γ/δ T cells can acquire DC phenotype and function (164). Furthermore, proinflammatory cytokines can endow human NK cells with ability to acquire antigen and to stimulate T cells (165). These observations suggest remarkable plasticity/redundancy in the system of antigen presenting cells (Fig. 3). Thus, the picture emerges in which monocytes yield all myeloid DCs while pDCs, γ/δ T cells and NK cells yield another set of cells with DC properties. The question to resolve is whether all DCs are equal and how are we going to define bone fide DCs (for example LCs) from cells that can acquire DC function under the environmental pressure (for example γ/δ T cells-derived DCs). A possible parameter for such distinction could be the extent of their professionalism measured by their capacity to prime naïve T cells. Furthermore, it will be important to determine which types of DCs do actually represent tissular DCs (myeloid).

The Rainbow of Myeloid DCs. This concept of plasticity/flexibility of the DC system is even further exemplified by monocytes and their response to environmental signals. Thus, monocytes can differentiate into either macrophages, which act as scavengers, or DCs that induce specific immune responses (166, 167). Different cytokines skew the *in vitro* differentiation of monocytes into DCs with different phenotypes and function (Fig. 4). Thus, when activated (for example by GM-CSF) monocytes encounter IL-4 they will yield IL4-DCs (143, 144, 168). By contrast, after encounter with IFN-α/β, TSLP, TNF, or IL-15, activated monocytes will differentiate into IFN-DCs (169–172), TSLP-DCs (173, 174), TNF-DCs (175) or IL15-DCs (176), respectively. Thus, rather than classical distinction of LCs and intDCs, we should consider myeloid DCs as a gradient (or rainbow) (Fig. 4). This spectrum of DCs represents immunostimulatory DCs. However, there exists a whole repertoire of DCs that exhibit immunoregulatory/tolerogenic functions, for example DCs generated by culturing monocytes with IL-10 in the presence of inflammatory cytokines such as GM-CSF or IFN-α (116, 177–179). These are important in the context of DCs role in maintaining peripheral tolerance. Thus, we view myeloid DCs as central cells for priming T cell immunity (Fig. 4). mDCs are polarized by other cells and their products including IFN-α from pDCs, IFN-γ from γ/δ T cells and NK cells, IL-4 and TNF from mast cells, IL-15 and TSLP from stromal cells, IL-10 from lymphocytes. These distinct DCs will induce distinct types of T cell immunity. The challenge for years to come will be to link these distinct DC phenotypes *in vitro* with a specific type of immune response and immune pathology *in vivo* as exemplified by TNF and IFN-α (180, 181) or by TSLP in allergic inflammation (173).

Distinct DC Subsets are Endowed with Distinct Functional Properties. Each of these DC subsets has common as well as unique biological functions, determined by a unique combination of cell-surface molecules and cytokines. Thus, *in vitro* experiments showed that LCs and interstitial DCs generated in cultures of $CD34^+$ hematopoietic progenitors differ in their capacity to activate lymphocytes: interstitial DCs induce the differentiation of naïve B cells

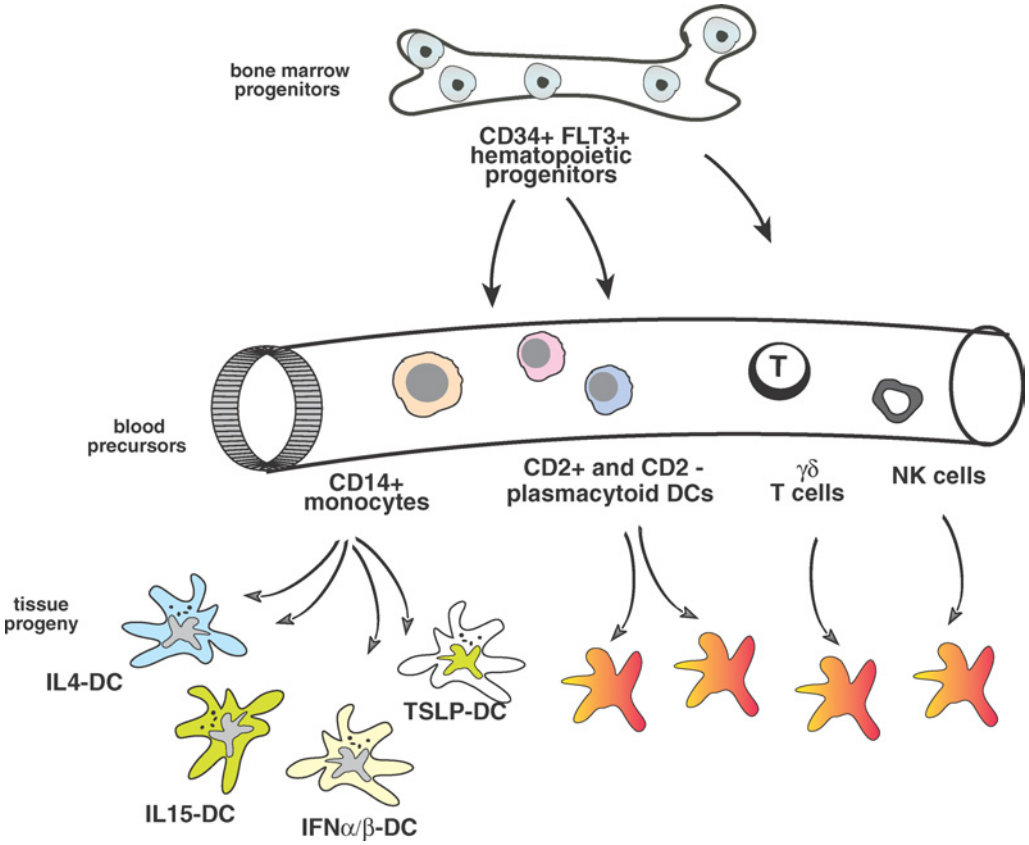

Fig. 3. Subsets of human dendritic cells. DC progenitors originate from bone marrow $CD34^{+}FLT3^{+}$ hematopoietic progenitor cells (HPCs). A myeloid pathway generates both Langerhans cells (LCs), found in stratified epithelia such as the skin, and interstitial (int)DCs, found in all other tissues. Another pathway generates plasmacytoid DCs (pDCs), which secrete large amounts of IFN-α/β after viral infection. Until recently monocytes and pDCs have been considered as major circulating DC precursor populations. Activated monocytes (for example via GM-CSF) yield DCs with different phenotype and function when exposed to different cytokines (IL4, IL15, IFNα/β, TSLP. . ..). CD2 expression in the human, and CD4 expression in the mouse, distinguishes pDCs subsets. Recent studies demonstrated that γ/δ T cells as well as NK cells can acquire DC phenotype and function under the environmental pressure. It remains to be determined how all these DCs relate to each other and which of them can prime naïve T cells, a seminal DC function.

into immunoglobulin-secreting plasma cells (145, 182), whereas LCs seem to be particularly efficient activators of cytotoxic $CD8^{+}$ T cells. They also differ in the cytokines that they secrete: only interstitial DCs produce IL-10; and their enzymatic activity (145, 182), which might be fundamental for the selection of peptides that will be presented to T cells. Indeed, different enzymes are likely to degrade a given antigen into different peptide repertoires, as recently shown for HIV nef protein (183). This will lead to different sets of pMHC complexes being presented and to distinct antigen-specific T-cell repertoires.

DC subsets express unique lectins (20), which at least partially account for the biological differences. Thus, LCs express Langerin, critical to the formation of Birbeck granules (184, 185). The role of these structures is not yet understood. The intDCs express DC-SIGN, involved in the interactions with T cells, DC migration, but also utilized by pathogens, for example HIV, to hijack the immune system (186–188). pDCs express yet another lectin BDCA2 (189, 190). TLRs are also differentially expressed. Such differential expression may permit specific *in vivo* targeting of DC subsets for induction of a desired type of immune responses, as recently demonstrated in mice by targeting DEC-205 (110, 111).

Importantly, we begin to understand the molecular pathways underlying differential responses of pDCs and mDCs to pathogens and/or pathogen derived factors. This

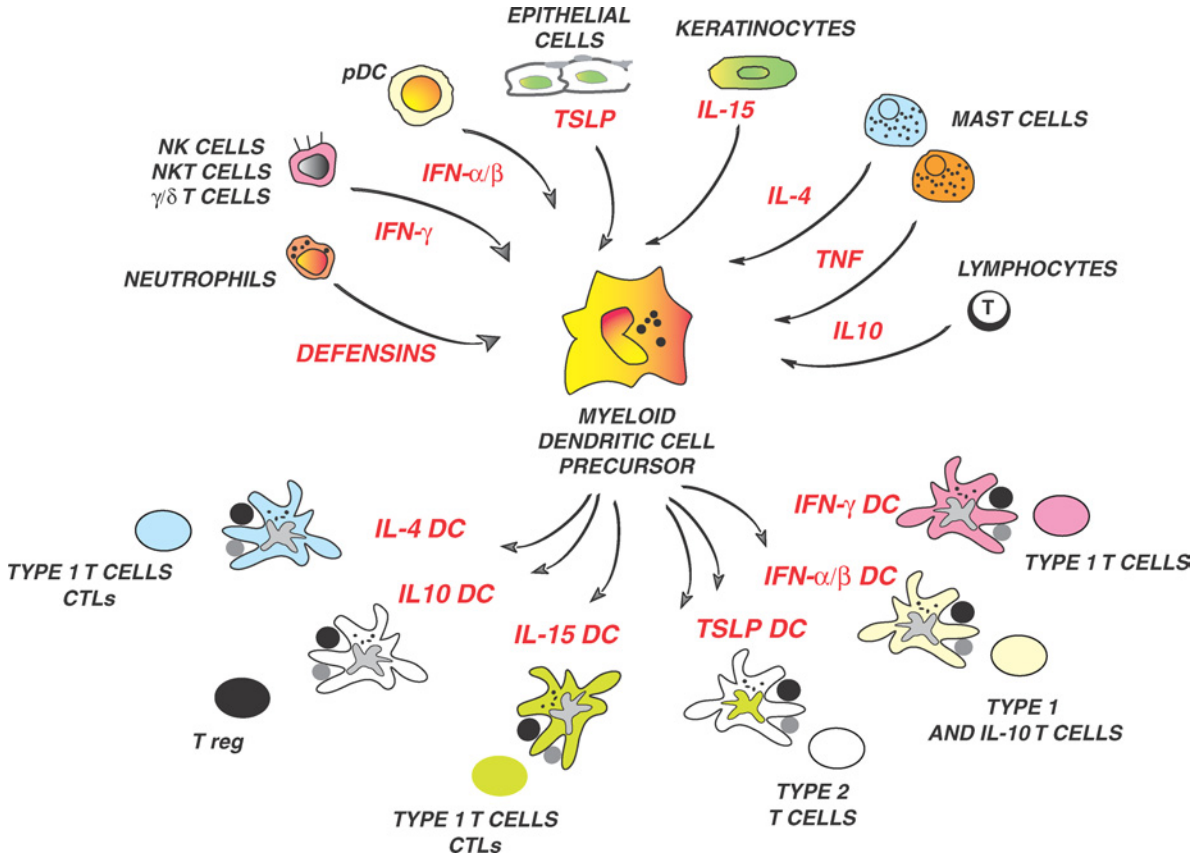

Fig. 4. Myeloid DCs as the information relay from the innate to the adaptive immune system. We propose a model in which the remarkable variety of observed T cell phenotypes could be explained by the plasticity of myeloid DCs. There, myeloid DC precursors yield different DCs upon encounter with different cells of the innate immune system and their products. Such imprinted DCs convey this information to immune effectors for example T cells. Each DC will trigger a unique type of T cell thereby permitting a broad functional repertoire. For example, IL-15 DCs are remarkably more efficient in priming and maturation of rare antigen-specific CTLs as compared to IL-4 DCs. TSLP-DCs induce CD4+T cells to differentiate into pro-inflammatory Type 2 cells secreting large amounts of IL-13 and TNF. Much remains to be done to establish the parameters of this model.

can be best illustrated by studies on mechanisms regulating type I interferon secretion. Thus, pDCs are recognized as a main source of type I interferon produced in response to viral (147) or CpG (191, 192) triggering. Recent studies demonstrated that IRF-7 is critical for IFNα/β secretion in response to both stimuli. However, IRF-7 activation in response to virus is MyD88 independent while response to CpG is dependent on both IRF-7 and MyD88 (193). It turns out that actually pDCs but not mDCs can direct CpG to endosomal compartments thereby allowing MyD88/IRF-7 activation and IFNα/β secretion (194).

Subsets of pDCs: Given the complexity of mDCs it is almost expected that pDCs will consist of subsets and/or demonstrate phenotypical and functional plasticity dependent on their microenvironment. The plasticity of pDCs is best illustrated by their distinct functions at two distinct stages of differentiation i.e., (i) ability of precursor pDC to secrete large amounts of IFN-α/β after viral infection (63, 147, 148); (ii) ability of mature pDC to activate and modulate T cell responses (195); and (iii) plasticity as demonstrated by induction of IFN-γ and IL-10 secreting T cells upon viral triggering and type 2 T cells upon activation with IL-3 and CD40 ligand (195). Recently the existence of pDCs subsets has been demonstrated. Thus, in the mouse expression of lymphoid-related genes (RAG1 and Ig rearrangement products (195)) or proteins (CD4 (196)) distinguishes between two subsets of pDCs. Although the functional consequence of RAG1 and Ig rearrangement products expression remains to be determined, $CD4^{neg}$ pDCs appear mainly responsible for migration to lymphoid tissue and IFN-α secretion upon exposure to CpG (196). Our results in humans demonstrate that CD2 expression distinguishes pDCs with common properties, such as IFN-α secretion, and unique functions, such as

the cytotoxic activity of $CD2^+$ pDCs against K562 cells (our own observations).

How DCs Interact with Other Cells of the Immune System

DCs regulate other lymphocytes as well including naïve (182) and memory (197) B cells, natural killer (NK) cells (198) and NKT cells (199).

Interaction with B cells. As discussed above myeloid DCs can prime naïve B cells. Several molecules have been shown to be involved in this process including IL-12, IL-6 (6) and, more recently BAFF/Blys (200–204), a molecule up-regulated by IFN-α. IFN-$\alpha\beta$ and IL-6 are also important in the differentiation of activated B cells into efficient Ig-secreting plasma cells upon exposure to virus-triggered pDCs (197). Strikingly, the plasma cells generated under these conditions express very high levels of CD38, similar to that of plasma cells isolated from lymphoid tissues. In contrast, plasma cells generated by culturing activated B cells with the T cell-derived cytokines, IL-2 and IL-10, though efficient Ig secretors, do not express high levels of CD38 (205). This suggests that IFN-$\alpha\beta$ may represent an important cytokine in the generation of tissular plasma cells. Indeed, studies in the mouse have also found that IFN-$\alpha\beta$ is an excellent adjuvant for humoral immunity (206). However, there may also be an indirect contribution of IFN-α to plasma cell differentiation through activation of myeloid DCs (207—210). Finally, differential activation of $CD4^+$T cells with B cell helper function by distinct DC subsets might play an important role in the induction of protective humoral immunity.

Cross-Talk with Innate Lymphocytes. DCs have a reciprocal interaction with natural killer (NK), NKT, and γ/δ T cells. Indeed, these innate immune effectors induce DC maturation through a combination of soluble and cell mediated signals (reviewed in (48)). In turn, mature DCs also stimulate NK (198, 211, 212), NKT (213), and γ/δ T cells (214, 215). These reciprocal interactions occur largely in the secondary lymphoid organs and are important for the amplification of type 1 T cell immunity. Thus, they extend the classical *ménage a trois* (5). It remains to be determined how DCs conditioning by the innate effector cells affects humoral immunity. Here again distinct DC subsets appear to differentially interact with innate lymphocytes. Thus, intDCs derived either from monocytes or from $CD34^+$HPCs directly stimulate NK-cell proliferation and cytotoxic function (216). On the contrary, LCs require exogenous cytokines to activate NK cells (216). Such specialization may have an important impact for *in vivo* DC targeting for vaccination.

DENDRITIC CELLS AND TOLERANCE

DCs are now thought to play a pivotal role in the control of tolerance (10, 11, 109, 217), both central and peripheral.

Central Tolerance

The thymus steadily produces thymocytes expressing newly assembled TCR some of which may be reactive with components of self. High affinity autoreactive thymocytes are eliminated on encountering self-MHC peptide (218–220). There is evidence that both thymic epithelial cells as well as mature DCs in the thymus may be involved in this process (221, 222). However, autoreactive T cells that are not deleted in the thymus need to be controlled in the periphery to prevent immune responses to self. Hence the need for peripheral tolerance that occurs in lymphoid organs.

Peripheral Tolerance Through DCs

There is now evidence that immature/steady state DCs control peripheral tolerance (reviewed in (109)). In the absence of inflammation, these DCs present tissue antigens to T cells in the absence of appropriate costimulation, leading to T-cell anergy or deletion (109), or the development of IL-10-producing, regulatory T cells as discussed earlier (131, 133). By suppressing a mandatory T cell help, DCs may also avoid a self-Ag specific and T-dependent B cell activation. The molecular mechanisms underlying the tolerogenic properties of peripheral DCs might involve: (a) lack of and/or inappropriate costimulation; (b) cell death induction by expression of Indoleamine 2,3-dioxygenase (IDO) which induces the catabolism of tryptophan, or by Fas/Fas-L interaction; (c) secretion of IL-10/TGF-β; and d) inhibitory receptors.

Inhibitory receptors: The effector function of immune cells is regulated by positive and negative signals provided through class-I recognition receptors (223–228). In humans, these are provided by KIR (Killer cell Immunoglobulin Receptor) and KLR (Killer cell Lectin like Receptor) family members of which interact specifically with certain HLA allotypes and class I related genes (229). A third class of receptors with both activating and inhibitory functions includes immunoglobulin like molecules (LILR, for Leukocyte Immunoglobulin like Receptors, also referred to as ILT, for Immunoglobulin Like Transcript). ILT family members are expressed on a wide variety of cells of both lymphoid and myeloid origin and can be divided into three groups according to function (230): 1) The inhibitory receptors with cytoplasmic ITIM motifs; 2) The activating receptors that associate with the ITAM containing FcR-gamma chain; and 3) a single member ILT6 which

does not contain a transmembrane region and might be secreted.

The two most extensively studied members are the inhibitory receptors ILT2 and ILT4. ILT2 is broadly expressed on all monocytes, DCs, most B-cells and subsets of T and NK cells (230). ILT4 is restricted to myeloid cells and expressed on all monocytes and some DC populations. Both ILT2 and ILT4 interact with multiple class-I alleles including HLA-G (231). Signaling through these molecules may be at least in part responsible for the immunosuppressive effects of HLA-G on antigen presenting cell function. Ligation of ILT4 on DCs by tetrameric HLA-G attenuates maturation in response to CD40L and reduce DC alloproliferative capacity (232). Expression of inhibitory ILT receptors appears to be a general feature of tolerogenic DCs (233). DCs treated with IL-10 specifically upregulate inhibitory ILT receptors (233) while blocking inhibitory ILT receptors lead to a restoration of the alloproliferative capacity in spite of reduced costimulatory molecule expression (179). This novel approach to understanding of DCs immunoregulatory function will be important for unraveling how pathogens evade immunity and for targeting of these pathways for therapy and for vaccination.

The importance of immunostimulatory and immunoregulatory signals has actually been already demonstrated in the Fc receptor (FcR) system (234). FcR share the same ligands, therefore the consequence of immune complex engagement on DC function depends on the balance between activating (ITAM) and inhibitory (ITIM) receptor types (236). In the steady state, this balance favors inhibition with the expression of ITIM containing FcγRIIb (235). It is shifted however in the presence of pro-inflammatory cytokines such as IFN-γ and TNF-α with the upregulation of FcγR3 and down regulation of RIIb, permitting immune complex mediated activation (235). Furthermore, the blockade of the inhibitory FcγR leads to spontaneous DC maturation with secretion of IL-12 p70 (237). Thus, inhibitory and stimulatory receptors expressed on DCs appear fundamental in determining tolerance or immunity.

DENDRITIC CELLS IN VACCINATION

Vaccination against infectious agents represents a success of immunology most particularly in diseases such as polio, measles, hepatitis B, and tetanus (238). However, many infectious diseases still evade the immune system, including chronic infections such as tuberculosis, malaria, and HIV. Further progress will be made through rational design based on our increased understanding of how the immune system works and how the induction of protective immunity is regulated. The same principle applies to cancer vaccines, most particularly as cancer is a chronic disease.

Ex vivo-generated and antigen-loaded DCs have now been used as vaccines to improve immunity in patients with cancer (239) and chronic HIV infection (240, 241), thus providing a "proof-of-principle" that DC vaccines can work. Previous reviews by us and others have emphasized the shortcomings of current vaccination protocols (242—245). Furthermore, we have emphasized throughout this article the key issues of DC biology that need to be considered for improved vaccination. Therefore, in summary, we will highlight a few points specific to vaccination in cancer.

Loading DC Vaccines with Antigen

Loading MHC class I and MHC class II molecules on DCs with peptides derived from defined antigens is the most commonly used strategy for DC vaccination (246, 247). Although this technique is important for "proof of concept" studies, the use of peptides has limitations: restriction to a given HLA type; the limited number of well characterized tumor-associated antigens (TAA); the relatively rapid turnover of exogenous peptide-MHC complexes resulting in comparatively low antigen presentation at the time the DC arrive into draining lymph node after injection; and, the induction of a restricted repertoire of T-cell clones, thus limiting the ability of the immune system to control tumor antigen variation. Thus, loading DCs with total antigen preparations and allowing "natural" processing and epitope selection is expected to improve efficacy as well as to allow the generation of a diverse immune response involving many clones of $CD4^+$ T cells and CTLs. These strategies include loading DCs with recombinant proteins, exosomes (248), viral vectors (249), plasmid DNA, RNA transfection (250), immune complexes (25) and antibodies specific for DC surface molecules (246, 251). Another technique involves exploiting the capacity of DCs to cross-priming discussed above (8, 252). This approach can be applied to load DC vaccines to elicit immunity against multiple antigens irrespectively of patients HLA type. Indeed, DCs cultured with killed allogeneic melanoma, prostate, and breast cancer cell lines prime naïve $CD8^+$ T cells against tumor antigens *in vitro* (252, 253). We have now vaccinated 20 patients with metastatic melanoma with autologous monocyte-derived DCs previously exposed to a killed allogeneic melanoma cell line (eight vaccines on a monthly basis). Vaccination has proved to be safe (no autoimmunity or other adverse events) and has led to the induction of melanoma-specific

T cell immunity. In two patients, this has resulted in long-lasting tumor regression (unpublished observations). These results warrant larger clinical studies.

Vaccination Frequency

We have found in 18 patients with metastatic melanoma that 4 vaccinations over 6 weeks with melanoma peptide-loaded CD34-DCs result in an increase in the number of melanoma-specific $CD8^+$ T cells in the blood as documented by IFN-γ ELISPOT (254, 255) and CTL assay (256). However, the melanoma-specific $CD8^+$ T-cell immunity in the blood was short-lived: all analyzed patients lost specific T cells detectable by direct ELISPOT and 4/9 patients lost all recall responses by 2 months after the last vaccination. Several explanations might be considered. T cells might migrate from the blood to peripheral tissue (tumor site) (257). Alternatively, the four bi-weekly vaccinations might have provided too frequent antigen stimulation for optimal T cell differentiation. Mouse and human studies of vaccination against infectious agents (258, 259) indicate that priming should be followed by a boost 4–6 weeks later for an optimal response. However, these rules may not apply to a chronic disease such as cancer. By analogy, chronic viral infections are associated with exhausted T cells owing to chronic antigen presentation (260, 261), and their reactivation through vaccination is likely to require different schedules. Accordingly, recent studies demonstrate that DC vaccination stimulates a pathway of accelerated generation of memory T cells that undergo vigorous secondary expansion in response to a variety of booster immunizations, leading to elevated numbers of effector and memory T cells and enhanced protective immunity (262).

Assessing Immune Efficacy

The ultimate parameters of efficacy of DC vaccines is the rate of objective tumor regression and improved survival. However, a detailed measurement of elicited T-cell and B-cell responses in the blood can provide important clues as to the efficacy of a given DC vaccine.

The Type of T Cell Immunity

Vaccine-specific T cell immunity has been classically measured by the quantity of tumor-antigen specific $CD8^+$ T cells (263). However, there is no defined threshold for how many T cells are sufficient to induce tumor regression. The elicited tumor antigen-specific T cells should be capable of cytokine production, proliferation on antigen re-exposure, migration to the tumor site and CTL function (264). Markers indicative of T-cell migration capacity include differential expression of CCR7 and CD45 isoforms: $CCR7^+$ $CD45RO^+$ T cells (central memory) will most likely migrate to lymph nodes, whereas the shift toward a $CCR7^-$ phenotype (effector memory) (265) should be associated with migration to the tissue.

Activation of Other Immune Effectors. $CD4^+$ T cells, NKT and NK cells, and B cells also need to be taken into account when analyzing vaccine specific immunity. In particular, $CD4^+$ T cells seem to be fundamental for priming long-lived $CD8^+$ T-cell memory (266–268). In fact, the lack of $CD4^+$T-cell activation in peptide-vaccination strategies might explain their limited efficacy in patients with cancer. Although a large number of circulating effector $CD8^+$ T cells might be elicited by such vaccines, in the absence of $CD4^+$ T-cell help, their quality might be compromised and the establishment of specific $CD8^+$ T-cell memory is unlikely (269). The induction of NKT cells, which kill a wide spectrum of tumor cells (270), or NK cells, which recognize MHC-class-I-deficient tumor cells (223), could be desirable, yet caution must be taken with regard to the cytokines that they produce. For example, IL-13-producing NKT cells may inhibit CTL-mediated tumor elimination and favor tumor progression (271).

Regulatory/Suppressor T cells

A major obstacle to the success of cancer vaccines, and possibly vaccines in chronic infections, including DCs might be the presence of regulatory/suppressor T cells and the demonstration that DCs regulate their expansion as discussed above. Indeed, a large body of experimental evidence shows that these T cells suppress anti-tumor immunity and that their removal allows tumor eradication (272, 273). An increased frequency of $CD4^+CD25^+$ T cells has been observed in the blood and tissues of patients with cancer (274–276). It is conceivable that distinct DC subsets and/or distinct DC maturation stimuli will have different capacities to induce regulatory T cells. This aspect needs to be explored further. Naturally occurring $CD4^+CD25^+$ suppressor T cells may be controlled by pretreatment of patients with drugs that can eliminate and/or control these cells, meaning that DC vaccination may be more effective when combined with other therapies.

CONCLUDING REMARKS

DCs are the critical decision-making cells in the immune response. DCs are an attractive target for therapeutic manipulation of the immune system to enhance otherwise insufficient immune responses to tumor antigens. However, the complexity of the DC system requires rational manipulation of DCs to achieve protective or

therapeutic immunity. Thus, further research is needed to analyze the immune responses induced in patients by distinct *ex vivo*-generated DC subsets activated via different pathways. The ultimate *ex vivo*-generated DC vaccine will be heterogeneous and composed of several subsets, each of which will target a specific immune effector. These *ex vivo* strategies should help identify the parameters for DC targeting *in vivo*, which represents the next step in the development of DC-based vaccination.

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