

Ins and Outs of Dendritic Cells

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Key Words

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Abstract

Dendritic cells (DC) are professional antigen-presenting cells which are strategically positioned at the boundaries between the inner and the outside world, in this way bridging innate and adaptive immunity. DC can initiate T cell responses against microbial pathogens and tumors due to their capacity to stimulate naïve T cells. The development of DC occurs in distinct stages. DC precursors develop in the bone marrow and home to a large variety of tissues. Immature DC capture antigen (Ag) and, following proinflammatory signals, migrate to the lymphoid organs where, after maturation, they present captured Ag to naïve T cells, thereby inducing differentiation of naïve T cells into effector T cells. An important cognate event in the development of cell-mediated immunity is the interaction between CD40 and CD40 ligand. Ligation of CD40 on DC by its ligand results in maturation of the DC. In addition to CD40 ligand (expressed by activated Th cells), inflammatory cytokines, bacterial components or Ag-Ab immune complexes can induce maturation of DC. Maturation of DC is crucial for the priming of efficient T cell responses and is characterized by a decreased Ag processing capacity, an increased cell surface expression of MHC and costimulatory molecules,

and rearrangement of cytoskeleton, adhesion molecules, and cytokine receptors. Mature DC migrate from peripheral tissues to secondary lymphoid organs, where T cell priming occurs. DC are not only critical in initiating T cell immunity, they also play a role in the induction of T cell tolerance and the regulation of the type of T cell response that is induced. Here we give an overview of the dendritic cell system.

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Introduction

Dendritic cells (DC) are professional antigen-presenting cells (APC) which are strategically positioned at the boundaries between the inner and the outside world, thus bridging innate and adaptive immunity [1]. Translocating antigen (Ag) from the periphery to the lymphoid niches is a pivotal function whereby DC initiate T cell responses against microbial pathogens and tumors due to their capacity to stimulate naïve T cells [2].

The development of DC is considered to occur in distinct stages. Hematopoietic pluripotent stem cells, under as yet unknown influences, constantly generate DC progenitors in the bone marrow, which give rise to circulating precursors in the blood [3]. These DC precursors home to a large variety of tissues where they reside as sentinel immature cells with high endocytic and phagocytic capacity [4, 5]. Immature DC capture Ag and, following proinflammatory signals that are often triggered

by infectious agents, migrate to the lymphoid organs where, after maturation, they present captured Ag to naïve T cells [4], thereby inducing differentiation of naïve CD4⁺ and CD8⁺ T cells into T helper (Th) cells and cytotoxic T lymphocytes (CTL), respectively. Ag-specific T cell activation requires the engagement of the T cell receptor/CD3 complex with the antigenic peptide presented by the major histocompatibility complex (MHC) molecules (signal 1), as well as the engagement of appropriate costimulatory receptors (signal 2) by costimulatory ligands on the APC.

An important cognate event in the development of cell-mediated immunity is the interaction between CD40 and CD40 ligand (CD40L). CD40 is fairly widely distributed and is expressed on B lymphocytes, monocytes and DC, but also on endothelial and epithelial cells [6]. The ligand of CD40 (CD40L or CD154) has a more restricted distribution, being mainly expressed by activated CD4⁺ T lymphocytes [7]. Ligation of CD40 on DC results in maturation of the DC [8], which is crucial for the priming of efficient T cell responses [9]. In the 'licence to kill' model [10–13], Th cells induce maturation of APC, thereby licencing them to directly activate CTL. In addition to Th cells, inflammatory cytokines [tumor necrosis factor (TNF)- α or interleukin (IL)-1], bacterial components (such as lipopolysaccharide, LPS) or Ag-Ab immune complexes (IC) can induce maturation of DC. Maturation of DC is characterized by a decreased Ag-processing capacity and an increased cell surface expression of MHC and costimulatory molecules [2, 4, 14]. In addition, rearrangement of cytoskeleton [15], adhesion molecules [16], and cytokine receptors [17] upon maturation allows DC to migrate from peripheral tissues to secondary lymphoid organs, where T cell priming occurs.

Although it is known that DC are critical in initiating T cell immunity, emerging evidence suggests that DC also play a role in the regulation of such responses. For example, distinct DC subsets can differentially regulate the Th1/Th2 balance in vivo [18–20] and in vitro [21]. Not only should the immune system attack that which is foreign or aberrant, it should also leave alone that which is neither, to avoid autoimmunity [4, 22]. Thus, DC constitute a complex system of cells that, under different microenvironmental conditions, can induce such contrasting states as immunity and tolerance [5].

A specialized DC system is required, since the initiation of T cell immunity is rather demanding [4]. Initially, peptides from infected cells located anywhere in the body must be found and recognized by T cells that circulate in the bloodstream. The amounts of specific Ag-MHC com-

plexes on tumors and infected cells are typically small, and must be recognized by rare T cell clones through a T cell receptor that has a low affinity. Moreover, infected cells and tumors frequently lack the costimulatory molecules that drive clonal expansion of the T cell, the production of cytokines, and development into killer cells. The DC system provides a powerful widely distributed cellular sentinel apparatus to solve these challenges. Here we give an overview of the properties of this DC system. Figure 1 schematically shows the central regulatory role of DC in the immune system.

DC Subsets

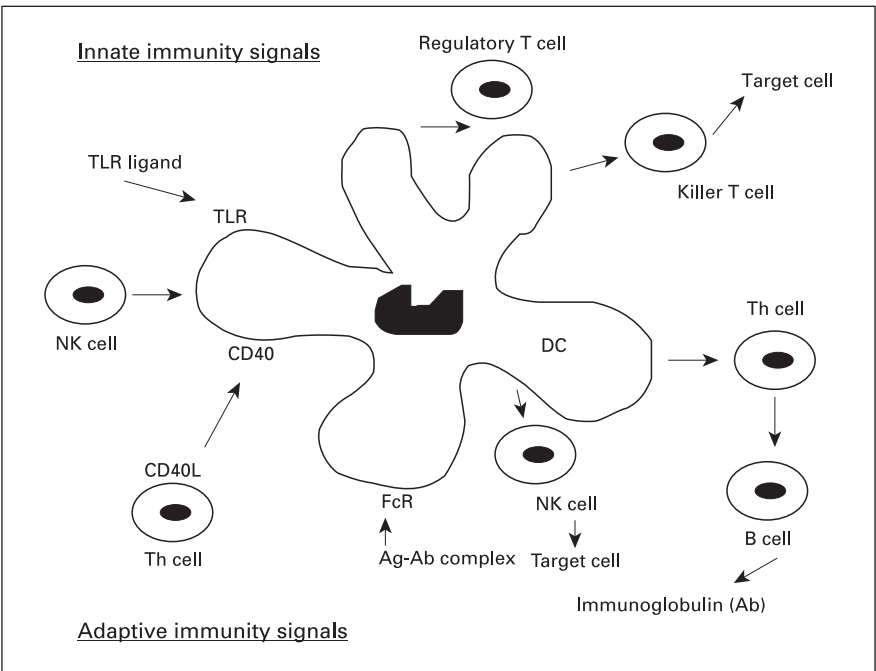
In the past, it has become generally accepted that DC are a distinct lineage of APC with potent capacity to induce primary T cell-mediated immune responses. However, accumulating evidence has demonstrated that the DC system bears much more plasticity than originally thought. DC should be looked at as a multilineage system of leukocytes with variable functions rather than a homogeneous cell type with predetermined functional properties.

Human DC Subsets

Two subsets of DC were identified in the human blood based on the expression of the β_2 integrin, CD11c [23]. Each of the subsets represents a small fraction (~0.3%) of the entire circulating blood leukocyte population [24]. More recent work has characterized these two subsets as belonging to the myeloid or lymphoid lineage and, although different denominations have been used, they can be defined as myeloid DC (M-DC) and plasmacytoid DC (P-DC) [25–28]. M-DC and P-DC differ in morphology, expression of markers and function. Both DC subsets (M-DC and P-DC) also share markers, such as adhesion molecules, costimulatory molecules, activation markers and inhibitory molecules. Table 1 shows surface marker profiles of these DC subsets.

M-DC are characterized by a monocytic morphology, possessing an irregular outline and a hyperlobulated nucleus. M-DC express myeloid markers like CD13, CD33, the β_2 integrin CD11c and low levels of the IL-3R α -chain CD123 [23, 29–31]. M-DC are able to present lipid Ag to T cells through expression of MHC class I-like molecules such as CD1a, b, c and d [32]. In contrast to P-DC precursors, M-DC precursors express high levels of the mannose receptor and can rapidly take up large amounts of polysaccharide Ag [33]. Peripheral blood monocytes give

Fig. 1. DC play a central regulatory role in the immune response. They receive signals from the innate immune system via surface receptors (TLR) that recognize pathogen-derived ligands. In addition, DC receive signals from natural killer (NK) cells. In turn, DC regulate the adaptive immune system by delivering positive or negative signals to T cells. CD4+ Th cells provide help to CD8+ killer T cells (CTL) and B lymphocytes. After differentiation to effector cells, CTL mediate eradication of target cells, whereas B lymphocytes produce Ab. After activation, NK cells kill their target cells. Th cells signal back to DC via upregulation of CD40L, which can bind to CD40 on the DC, thereby inducing maturation of the DC. B lymphocytes signal back to the DC via the production of Ab, which, after binding their Ag, can bind to receptors for the Fc domain of Ig (FcR), thereby inducing maturation of the DC. Thus, the interaction of DC with the innate and adaptive immune system is a dialogue rather than a monologue, depending on reciprocal interactions.



rise to immature M-DC after culturing with granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-4 [34, 35]. Functionally, M-DC capture Ag in the peripheral tissues by both phagocytosis and macropinocytosis [28]. After Ag uptake, these immature M-DC migrate to lymph nodes. Stimulation with CD40L or endotoxin [8, 36] induces the maturation of M-DC. Based on their ability to produce IL-12 in response to LPS and prostaglandin (PG) or CD40L stimulation [37, 38], M-DC were regarded as a Th1-driving APC type that induced the differentiation of naïve CD8+ T cells into CTL [39], and therefore designated as DC1 [18, 19, 21]. However, several in vitro and in vivo studies show that M-DC are potent inducers of both Th1 and Th2 cytokines in naïve Th cells [40–46]. An essential discriminative factor appears not to be the ability to produce IL-12 per se, but rather the level of IL-12 produced [42, 47]. Thus, by inducing IL-12 production in DC, bacteria, bacterial compounds, or exogenous interferon (IFN)- γ , e.g. derived from activated bystander memory T cells or NK cells, will promote the development of Th1 responses [42, 47]. Conversely, Th2 responses will be facilitated by microenvironmental factors that inhibit the production of IL-12 by DC, such as IL-10 [48] and PGE₂ [42, 47, 49, 50]. Neither a Th1- nor a Th2-inducing function is an intrinsic attribute of human M-DC, but appears to depend on environmental instruction [51].

Table 1. Expression of surface markers on human and mouse DC subsets

Surface Ag	M-DC		P-DC		L-DC
	human	mouse	human	mouse	mouse
CD4	+	+ or –	++	±	–
CD11a	++	++	+	++	++
CD11c	++	++	–	±	++
CD13	++		–		
CD18			++		
CD31	++		++	–	
CD33	++		–		
CD36	+		++	+	
CD38			++	++	
CD40	++	+	++	+	++
CD44			++	++	
CD45RO	++		–	–	
CD45RA	–		++	++	
CD54	++	++	++	++	++
CD123	±		++	+	
CCR5	++				
CXCR3	–		++		
ILT1	++		–		
ILT3	++		++		
MHC class II	++	++	++	++	++

Expression levels are indicated: – = absent; ± = low; + = intermediate; ++ = high. When nothing is mentioned in the table, no information about expression of the indicated marker on that DC subset was found in the literature. M-DC = Myeloid DC, P-DC = plasmacytoid DC, L-DC = lymphoid DC.

P-DC, derived from a lymphoid lineage [26, 52], have a morphology resembling plasma cells possessing a rounded morphology with an oval or indented nucleus and a prominent perinuclear pale zone. P-DC are devoid of myeloid markers such as CD13 and CD33 and express high levels of CD123 (the α -chain of the IL-3R), CXC chemokine receptor 3 (CXCR3), and MHC class II molecules [28, 30, 33, 52, 53]. In contrast to M-DC, P-DC do not express CD11c [53]. In addition, P-DC specifically express a unique lectin recognized by the monoclonal Ab BDCA2. Ag binding to this lectin is efficiently taken up, processed and presented to T cells [54]. P-DC from blood or tonsils give rise to a distinct type of immature DC after culture with IL-3 [31, 33]. These cells differentiate into mature DC after CD40L stimulation [21] and induce T cell responses with a Th2 phenotype [31, 33, 53]. In comparison to M-DC, P-DC produce very low amounts of IL-12, upon CD40L stimulation [55]. Without IL-3 in the medium they die rapidly by apoptosis. CD40L together with IL-3 promotes the differentiation of P-DC into DC that express little CD13, CD33 and CD1a [33]. P-DC were designated as DC2 [53, 21] for their ability to induce Th2-type responses [31, 33, 53]. Recently, however, it was discovered that upon exposure to viruses, P-DC produce very high levels of IFN- α , a type I IFN [26, 53]. In human beings type I IFNs are Th1-polarizing cytokines and, indeed, after exposure to viruses, P-DC have been shown to induce potent Th1 responses [53, 56, 57]. Moreover, it has been shown that P-DC induce IL-10-producing CD8+ T cells [39]. Thus, P-DC are shown to be able to induce both Th1 and Th2 responses under different conditions [18, 41, 58].

The combination of the abilities to pick up Ag, carry it from peripheral tissues to the draining lymph nodes, and stimulate naïve Th cells efficiently is a unique property of M-DC [18, 59]. In contrast, P-DC are inefficient in Ag capture by phagocytosis and macropinocytosis at all maturation stages [33]. M-DC are located at sites of pathogen entry such as skin and mucosal tissues. P-DC on the other hand are mainly located within the T cell areas of lymphoid tissues under normal physiological conditions [33] and therefore may be specialized to recognize self-Ag or blood-borne pathogens such as viruses [28].

Murine DC Subsets

In mice, splenic DC were originally separated into CD8 α +CD11b- and CD8 α -CD11b+ subpopulations, each expressing CD11c [60]. CD8 expression on DC is in the form of an $\alpha\alpha$ -homodimer rather than the $\alpha\beta$ -het-

erodimer that is typical of T cells. All CD8+ mouse DC were thought to derive from lymphoid-restricted precursors, and all CD8- DC to derive from myeloid precursors, leading to the terms 'lymphoid' and 'myeloid' DC (L-DC and M-DC). However, this concept has proven not to be correct. The isolation of lymphoid and myeloid precursor cells from bone marrow showed that both precursors could produce all the mature splenic and thymic DC types [61–64]. Studies of the kinetics of DC subset labelling after continuous administration of a DNA precursor to mice have indicated that DC subtypes are not products of each other and that all subtypes are short-lived [65]. A degree of DC sublineage commitment must therefore occur downstream of the early hemopoietic precursors. 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 CD8 α - DC, mice bearing a mutant Ikaros gene are deficient in CD8 α + DC [66, 67]. Surface marker profiles of DC subsets are shown in table 1.

In contrast to CD8 α - DC, CD8 α + DC express CD1d and lectin DEC-205. CD8 α + DC are concentrated in the T cell areas in the spleen. CD8 α + DC have the ability to produce large amounts of IL-12, induce Th1 responses [19, 20, 68], and cross-prime CD8+ T cells [69–71]. CD8 α - DC are concentrated in the marginal zones of the spleen, but migrate into the T cell zones on stimulation with microbial products [72, 73]. CD8 α - DC do not have the ability to produce large amounts of IL-12. They preferentially induce Th2 responses and do not have the ability to cross-prime CD8+ T cells [74]. Recently, the CD8 α -CD11b+ DC subset was further subdivided into a CD4+ subset and a CD4- subset [60]. The functional differences between these two subsets are currently unknown.

More recently a third subset of murine DC has been identified, the murine P-DC. P-DC express Ly6C and B220 and have low levels of CD11c. The mouse homolog of P-DC was purified directly from lymphoid organs [75–78]. Mouse P-DC share with human P-DC the characteristic plasmacytoid appearance, the high IFN- α production in response to viral and other microbial stimuli as well as the expression of many surface markers. However, they lack CD123 expression and express B220, CD11c and Gr-1.

In mice the functional plasticity of DC is mostly dependent on the type of pathogen and on the tissue microenvironment. The fungus *Candida albicans* for example stimulates DC to produce IL-12 and induce Th1 responses at the yeast stage. At the hyphae stage, however, *C. albicans* stimulates DC to produce IL-4 and induce Th2

responses [79]. Furthermore, DC isolated from Peyer's patches [80], respiratory tract [45] and liver [81] preferentially induce Th2 differentiation. In contrast, DC from the spleen preferentially induce Th1 differentiation. Recently it has been shown that, in addition to P-DC, M-DC also produce IFN- α upon infection with viruses [82]. These differences may reflect differences in the tissue cytokine microenvironment and exposure to particular pathogens, as well as differences in the lineage origin of different tissue DC. Since DC produce proinflammatory cytokines, chemokines and IL-12 only for brief periods of time, and at defined stages of maturation, this might also influence the outcome of the response [83].

Ag Processing

Presentation of Endocytosed Ag in MHC Class II

To sample their environment in peripheral tissues, DC constitutively macropinocytose extracellular fluid and take up Ag via phagocytosis and receptor-mediated endocytosis. DC express several receptors that facilitate the internalization and presentation of Ag, including C-type lectin receptors such as the mannose receptor [84] and DEC205 [85], as well as receptors for the Fc domain of immunoglobulins (Ig; Fc γ R and Fc ϵ R, which bind the Fc domain of IgG and IgE, respectively). Furthermore, DC express specific receptors for heat shock proteins (hsp) such as gp96 and hsp 70, mediating the internalization of hsp-peptide complexes [86, 87].

After capture and internalization, Ag are proteolytically processed into 12- to 25-amino-acid-long fragments that bind to MHC class II molecules for presentation to CD4⁺ T lymphocytes [88]. It is currently believed that loading of MHC class II molecules with peptide occurs in a specialized compartment that is located at the intersection of the biosynthetic route of the MHC class II molecules and the endocytic pathway. The MHC class II molecule consists of an α - and a β -chain that associate in the endoplasmic reticulum (ER) with the 31- to 33-kD invariant chain (Ii). The $\alpha\beta$ Ii complex exists as a nine-subunit transmembrane protein that contains three $\alpha\beta$ -dimers associated with an Ii trimer. A short internal fragment of the Ii, called the class II-associated invariant chain peptide (CLIP), occludes or closes the class II peptide-binding site, thereby avoiding presentation of peptides present in the ER. The $\alpha\beta$ Ii trimers are transported via the Golgi stacks to the trans-Golgi reticulum. There the majority of all the $\alpha\beta$ Ii trimers are sorted to specialized endocytic compartments, which are enriched for MHC class II

molecules. These compartments are called MIIC (MHC class II-enriched compartments). The majority of intracellular MHC class II molecules reside in those MIIC. MIIC are multivesicular body-like or contain membrane sheets similar to those present in lysosomes of other cell types [89–91] and contain the lysosomal marker proteins Lamp-1/2 and CD63. $\alpha\beta$ Ii complexes are targeted to endocytic compartments through a signal in the cytoplasmic tail of Ii. Just before arriving or at this compartment the Ii is gradually degraded by proteolytic cleavage, leaving only CLIP in the peptide-binding groove of MHC class II dimers. Then the nonpolymorphic MHC class II molecules HLA-DM in humans (H2-M in the mouse) and HLA-DO (H2-O in the mouse) catalyze the exchange of CLIP for antigenic peptides [92–94]. Finally, peptide-loaded MHC molecules are delivered to the cell surface [95, 96]. Alternatively, MHC-peptide complex formation can involve internalized MHC class II molecules, which exchange their associated peptides in early endocytic compartments before returning to the cell surface [5, 30].

Exosomes

MHC class II molecules are transported from MIIC to the plasma membrane via unknown pathways. It was shown that MIIC can fuse directly with the plasma membrane [97]. This process is responsible for delivery of part of the intracellular MHC class II to the cell surface. MIIC contain characteristic internal membrane vesicles with MHC class II exposed on their surface. Fusion of MIIC with the plasma membrane results in the release of these MHC class II-expressing vesicles, named exosomes. In addition to MHC class II molecules, exosomes have now been shown to express other proteins which play a role in Ag presentation (MHC class I and costimulatory molecules) [98–100]. In contrast, Ii or HLA-DM, two other important players in the process of MHC class II-restricted Ag presentation, that are very abundant in endosomes and lysosomes, are not detected in exosomes. Therefore, a highly specific mechanism of protein segregation within multivesicular endosomes, between the internal vesicles and the limiting external endosomal membrane, results in the selective enrichment of certain proteins in exosomes. In addition to MHC class I and II and B7.2, exosomes express integrins, Ig family members, tetraspanins, heat shock proteins, cytoskeletal proteins and proteins that play a role in membrane transport and fusion, signal transduction or metabolism [98–103]. The oligomer-forming tetraspan proteins have been implicated in Ag presentation, T cell signaling, T cell activation, cell motility and adhesion, and form complexes not only with one

another [104–106], but also with HLA-DR [104, 107], MHC class I [108], integrins [106] and the T cell coreceptors CD4 and CD8 [109]. Such large protein networks might limit the diffusion of molecules such as MHC class II and HLA-DM, thereby facilitating intermolecular interactions. Furthermore, exosomes are enriched in cholesterol, sphingomyelin and ganglioside, lipids that are typically enriched in detergent-resistant membranes or rafts. Recruitment of membrane proteins from the MIIC-limiting membrane to the internal vesicles may occur via their incorporation into tetraspanin-containing rafts [100]. The definition by proteomic studies of a subset of cellular proteins that are targeted specifically to exosomes clearly shows that exosomes are distinct from the microvesicles that are produced by apoptotic cells and that they are only secreted by living cells [99, 102].

Although the physiological targets and functions of APC-derived exosomes remain largely to be resolved, follicular DC (FDC; present in the germinal centers of secondary lymphoid organs) have recently been shown to bind B lymphocyte-derived exosomes at their surface, which supports the notion that APC-derived exosomes play an immunoregulatory role [110]. Whereas Ag-Ab complexes retained by FDC are pivotal for selection of high-affinity B lymphocytes, exosomes docked on FDC might select and recruit specific Th cells. In this way, FDC might sieve and facilitate the interaction between matching B and T cells, ultimately leading to isotype switching and differentiation of B lymphocytes into plasma cells or memory cells. Furthermore, in vitro and in vivo studies with isolated exosomes showed that Ag-loaded exosomes stimulate murine CD4⁺ T cell clones [97], that tumor-peptide-loaded exosomes induce rejection of established mouse tumors [111] and that exosomes bearing an H-Y peptide activate specific naïve T cells in vivo [112]. The presence of DC is required in vitro to obtain efficient Ag presentation to T cells by exosomes [112, 113]. Although exosomes are immunogenic in the experimental settings reported above, it remains to be determined whether T cell stimulation by APC-derived exosomes might also induce tolerance. Exosomes are produced not only by APC, but also by reticulocytes (to discard membrane proteins) [114, 115], various hematopoietic cells [97, 102, 111, 116–118], tumor cells [103, 119] and epithelial cells [120, 121]. Indeed, exosomes (or ‘tolerosomes’) produced by OVA-loaded rat epithelial cells induced some degree of Ag-specific tolerance [122].

Exosomes may play a role in spreading Ag-specific signals, by exchanging both Ag and MHC/peptide complexes between different DC, thereby functioning in in-

tercellular communication between cells of the immune system. The use of small vesicles for communication/signaling between cells is also exploited in the central nervous system, where synaptic vesicles are secreted by one neuron, to signal to the other.

Regardless of their putative physiological role, exosomes have the potential to be used as tools for vaccination. Induction of tumor protection by exosomes secreted by bone marrow-derived DC pulsed with tumor peptides has been shown [111]. On the basis of these results, the use of exosomes for the immunotherapy of cancer patients was undertaken. Two phase I trials were started recently, one using MAGE3-epitope-loaded DC-derived exosomes in patients with metastatic melanoma, the other using DC-derived MAGE3-peptide-loaded exosomes in unresectable non-small-cell lung carcinoma patients. Exosomes have been proven to be a safe vaccine in these patients [123]. In addition to DC-derived exosomes, tumor-derived exosomes are used in clinical trials. Tumor-derived exosomes contain tumor Ag, which are presented to MHC class I-restricted T cell clones in vitro, after exosome loading onto DC [103]. Recently, malignant effusions from cancer patients have been found to contain exosomes. Tumor-derived exosomes were isolated from ascites of patients with peritoneal or pleural carcinomatosis associated with ascitis or pleural effusions with tumor cells in the biological fluid. These exosomes have been shown to deliver tumor Ag to monocyte-derived DC in vitro. Lymphocytes specific to the tumor could be expanded from peripheral blood cells by pulsing DC with ascites exosomes [119].

Presentation of Endogenous Ag in MHC Class I

In higher organisms, MHC class I molecules are expressed on the surface of virtually all nucleated cells, where they present Ag to CD8⁺ T cells [124, 125]. Shortly after synthesis in the ER the MHC class I heavy chain associates noncovalently with a nonpolymorphic light chain, termed β_2 -microglobulin, and MHC class I molecules are loaded with peptide in the ER. Most of these peptides are derived from cytosolic proteins. Ag processing starts by ubiquitin conjugation in the cytosol [124, 125]. Ubiquitinated proteins are degraded by the proteasome, a multisubunit proteolytic complex, in an ATP-dependent fashion. After peptides are generated in the cytosol, they are translocated across the ER membrane by the transporter associated with Ag presentation (TAP) [126] and loaded onto newly synthesized MHC class I molecules within the ER [124, 127]. TAP is a heterodimer composed of two subunits, TAP1 and TAP2, each con-

taining an N-terminal hydrophobic region with multiple predicted transmembrane domains, and a cytosolic C-terminal ATP-binding domain. Expression of both TAP1 and TAP2 is required to get efficient binding, certain peptides bind preferentially to TAP1 while others bind preferentially to TAP2. Peptide binding is ATP-independent, while translocation is ATP-dependent [124].

The degradation of cytosolic Ag is an essential step in the generation of most MHC class I-presented epitopes. Cytosolic protein degradation is highly specific and tightly regulated to prevent nonspecific destruction of essential self-proteins. Degradation of cytosolic Ag and generation of MHC class I-associated epitopes should be rapid, to detect rapidly replicating pathogens, and efficient, to present sufficient numbers of epitopes from small amounts of Ag. While efficient peptide generation is desirable, the protein degradation pathway must remain sufficiently general to accommodate a plethora of foreign Ag [124].

'Cross-Presentation' of Exogenous Ag in MHC Class I

In addition to an exogenous pathway for processing of MHC class II-restricted Ag and an endogenous pathway for MHC class I-restricted Ag, DC have a specialized capacity to process exogenous Ag into the MHC class I pathway [128–130]. This function, known as cross-presentation, provides the immune system with an important mechanism for generating immunity to pathogens that avoid professional APC (cross-priming) and tolerance to self-Ag that are not expressed by the APC (cross-tolerance) [131, 132]. Induction of T cell tolerance by cross-presentation of cellular Ag occurs via induction of deletion [133–137] or anergy [22, 135, 137].

Two routes for the exogenous MHC class I pathway have been described and recently a third route was discovered. These routes are described below. A TAP-independent pathway in which Ag is most likely hydrolyzed in endosomes and loaded onto recycling MHC class I molecules present in these compartments has been shown [30, 138]. Another possible route for loading of peptides is a phagosome-to-cytosol pathway that is proteasome and TAP-dependent, resulting in peptide loading in the ER lumen and transport of peptide-loaded MHC class I molecules by the secretory pathway [30, 128, 129, 139]. Recently it was shown that soon after or during formation, phagosomes fuse with the ER. After export of phagocytosed Ag to the cytosol and degradation by associated proteasomes, peptides are rapidly translocated by TAP into the lumen of the same phagosomes, before loading on phagosomal MHC class I molecules [140–142]. This latter

pathway is a completely new cross-presentation route, since it is TAP-dependent (in contrast to the first-mentioned pathway) and peptide-loading takes place in the phagosome lumen instead of the ER lumen (in contrast to the second-mentioned pathway). Translocation to the cytosol is selective in the sense that there is no gross disruption of lysosome compartments and translocation is size selective [143]. The proteasome- and TAP-dependent pathway is thought to be involved in immune responses against transplantation Ag [144], particulate Ag [128], tumors [145], and viruses [126]. It is also operative in the development of tolerance [131]. Presentation of exogenous Ag in MHC class I in vitro can be improved by complexing Ag to beads [146] or heat shock proteins, by providing Ag in bacteria [147], by administering Ag in apoptotic cells or by providing Ag in the form of Ag-Ab IC [139, 148].

The relative contribution of the three different routes for peptide loading onto MHC class I molecules in vivo is unclear. In vivo cross-priming against tumor Ag requires TAP, suggesting that in this case the classic phagosome-cytosol-ER pathway or the ER-phagosome fusion pathway is predominant [149].

The source of cross-priming Ag can be either cellular protein, peptides, heat shock protein or combinations of these [150–154].

Environmental Signals for DC Maturation

Immature versus Mature DC

Recent studies have demonstrated a remarkable functional plasticity of a given DC subset to induce different types of T cell responses depending on the type of invading pathogens. Cells of the innate immune system recognize pathogen-specific molecular patterns (PAMP) using Toll-like receptors (TLR). The responsiveness to a given PAMP has been linked to the expression of a particular TLR. PAMP, such as LPS, bacterial DNA, and double-stranded RNA (dsRNA), induce DC maturation [30, 155]. Moreover, DC maturation can be induced by T cell-derived signals, such as CD40L [8, 36, 156], or by Ag-Ab complexes [139, 148, 157]. Furthermore, the balance between proinflammatory and anti-inflammatory signals in the local microenvironment, including TNF- α , IL-1, IL-6, IL-10, TGF- β , and PG, play a role in the maturation process [5, 49]. All these signals can induce immature DC to mature and migrate to the draining lymph nodes where they present captured Ag to naïve T lymphocytes. This is one of the ways in which DC bridge innate and adaptive immunity. DC maturation is gener-

Table 2. Expression of TLR on human and mouse DC subsets

TLR	Ligand (PAMP)	M-DC ^a	P-DC ^b	L-DC ^c
TLR1		++/++	++/++	++
TLR2	Peptidoglycan/mycobacteria\ Lipoteichoic acids	++/++	-/+	++
TLR3	Poly I:C	++/+	-/+	+
TLR4	LPS/lipoteichoic acids	++/+	-/+	++
TLR5	Flagellin	++/++	-/+	-
TLR6		+/++	+/++	+
TLR7	Imiquidazoquinolines\ssRNA	-/++	+/++	-
TLR8		+/++	-/++	++
TLR9	Unmethylated CpG DNA	-/++	+/++	++
TLR10		-/	±/	

Expression levels are indicated as described in the footnote of table 1. Ligands for the different TLR are indicated.

^a Expression on human M-DC/expression on murine M-DC.

^b Expression on human P-DC/expression on murine P-DC.

^c Expression on murine lymphoid DC (L-DC).

ally seen in response to pathogens or hallmarks of their presence.

Immature DC are specialized in capturing and processing Ag to form MHC peptide complexes. DC maturation is associated with decreased Ag uptake and acquisition of the capacity to efficiently present Ag and prime T cell responses. Maturation may be seen as a physiological response to infection with profound implications for T cell immunity. Maturation of DC is required for efficient induction of primary T cell responses. The efficacy of DC in T cell binding and activation cannot be attributed to a single specific molecule, but merely is the result of quantitative effects and their regulation. The DC maturation process is associated with several coordinated events such as loss of endocytic/phagocytic receptors, upregulation of MHC molecules and costimulatory molecules (e.g. CD40, CD58, CD80, CD86, OX40-L, CD70, 4-1BB-L), change in adhesion molecule and cytokine receptor expression and cytokine production, change in morphology, shift in lysosomal compartments with downregulation of CD68 and upregulation of DC-lysosome-associated membrane protein (DC-LAMP), and change in MHC class II-enriched compartments (MIIC) [5, 158, 159]. Depending on the conditions, DC can stimulate the outgrowth and activation of a variety of T cells, which affect the immune response differently. In response to maturation signals DC upregulate CCR7, a homing receptor, that facilitates migration to the lymph node [160–162].

Innate Immunity Maturation Signals

Innate immune recognition of invading pathogens is mediated by a set of receptors that have evolved to recognize conserved molecular patterns shared by a large group of pathogens [163]. Accumulating evidence has shown that this recognition can be attributed mainly to the TLR family [37, 164–166]. TLR are ancient microbial pattern recognition receptors highly conserved from *Drosophila* to humans [164, 167, 168]. Human Toll/IL-1 receptor-like proteins, which are homologues of the *Drosophila* Toll molecule, were identified. TLR are type I transmembrane proteins that have a leucine-rich repeat domain in the extracellular region and cytoplasmic domains, the Toll/IL-1 receptor homology domain (TIR) [169], that share sequence similarity with the cytoplasmic domain of the human IL-1 receptor. The TLR molecules mediate signaling through the adaptor molecules MyD88, IL-1 receptor-associated kinase (IRAK), and TNF receptor-associated factor 6 (TRAF6) [30, 170, 171]. This activates the NF-κB pathway and mitogen-activated protein kinases (MAPK) [30, 172, 173], thereby regulating the expression of the costimulatory molecule CD80 (B7.1) as well as the expression of a number of inflammatory cytokines [174]. In particular, induction of IL-12 in response to microbial lipoproteins is mediated by TLR [175]. To date, ten human and nine murine transmembrane proteins have been shown to belong to the mammalian TLR family. The two DC subsets of human peripheral blood, M-DC and P-DC, are demonstrated to express a different set of TLR. M-DC express all TLR except TLR7 and TLR9, which are selectively expressed by P-DC [37, 165, 176]. P-DC in contrast do not express TLR3. In contrast to the dichotomy in TLR expression on P-DC and M-DC in humans, mRNA for most TLR is expressed at similar levels by murine splenic DC subtypes, including P-DC. TLR3 is preferentially expressed by L-DC, while TLR5 and TLR7 are absent from this subset [177]. Table 2 shows the different TLR, their ligands and their expression patterns on human and mouse DC subsets. Microbial molecules that trigger TLR2, TLR3, TLR4, TLR5 and TLR9 have been identified.

Peptidoglycan and mycobacteria bind to and signal via TLR2 [178–181].

Polyinosine-polycytidylic acid (poly I:C), a viral dsRNA-like molecule, signals through TLR3. dsRNA is able to differentiate and activate DC. This results in a rapid production of type I IFN leading to expression of MxA, a protein that protects the DC from the lethal effect of the virus [182]. Upon exposure to LPS the expression of TLR3 is inhibited. This might represent a regulatory

mechanism after DC have encountered pathogens [176]. Poly I:C induces stable maturation of human DC with high expression levels of HLA-DR, CD86, and the DC maturation marker CD83 [183]. In addition, poly I:C-treated DC downregulate pinocytosis, produce high levels of IL-12 and low levels of IL-10, induce strong T cell proliferation, and effectively present peptide Ag to MHC class I-restricted CTL.

LPS from Gram-negative bacteria signals through TLR4. LPS is probably the most powerful microbial stimulant of innate immune responses. LPS can provoke a variety of immunostimulatory responses, for example production of inflammatory cytokines such as IL-12, TNF- α and IL-6 [57], and inflammatory effector substances such as nitric oxide [184]. These biological activities can be ascribed to the lipid A portion of LPS. TLR4 is a part of trimolecular LPS receptor complex [185, 186] that contains two coreceptors: MD-2, which is required for TLR4 function [175, 187, 188], and CD14 [186], which is a high-affinity GPI-linked LPS-binding protein. The structure of the proinflammatory component of LPS, lipid A [186], varies between bacteria of different species. Downstream from TLR4, MyD88 has been identified as a mediator of LPS signal transduction [189].

Mammalian TLR5 recognizes bacterial flagellin from both Gram-positive and Gram-negative bacteria [164, 169]. Activation of this receptor mobilizes the nuclear factor NF- κ B and stimulates TNF- α production.

Bacterial DNA containing unmethylated CpG motifs signals through TLR9, resulting in the production of IFN- α [164]. The key feature that distinguishes mammalian from bacterial DNA is that CpG motifs in bacterial DNA are unmethylated [190]. Thus, the vertebrate immune system has evolved a specific TLR that distinguishes bacterial DNA from self-DNA [171]. Synthetic CpG-containing oligodeoxynucleotide (CpG-ODN) mimics the stimulatory effect of bacterial DNA [170]. CpG-(ODN) acts as an adjuvant for DC and induces Th1-type responses most probably by the direct induction of IL-12 and upregulation of costimulatory molecules [190]. In humans, TLR9 is selectively expressed on P-DC [37, 55, 165]. CpG DNA can induce cytokine production from P-DC, but not from M-DC [165, 191]. CpG DNA must be internalized in order to stimulate cells. The cellular uptake of CpG is presumably followed by their interaction with an intracellular receptor. The intracellular location of the CpG receptor suggests that immune recognition of CpG DNA may have evolved as a defense against intracellular bacteria, viruses, and retroviruses.

Thus, pathogen recognition by TLR provokes rapid activation of innate immunity by inducing production of proinflammatory cytokines and upregulation of costimulatory molecules [165, 174]. Activated innate immunity subsequently leads to effective adaptive immunity. In this regard, the TLR are considered to be adjuvant receptors. Different TLR can exert distinct, but overlapping sets of biological effects [165]. TLR share a number of common signaling features such as IRAK and MyD88, but also exhibit substantial differences in cofactor requirements that result in relatively unique signaling mechanisms. Furthermore, it is likely that TLR cross talk has a profound impact on the ability of a cell to respond over a protracted period to diverse microbial products [192].

Adaptive Immunity Maturation Signals

CD40-CD40L Interactions

CD40 is a member of the TNF-R superfamily that plays a critical role in both humoral and cellular immune responses [10, 13, 193, 194]. It was initially shown to be constitutively expressed on the majority of B cells but has since been found to be expressed on a wide range of cells, including epithelial and endothelial cells and on all APC [195–197]. The natural ligand for CD40, CD154/CD40L, is a trimeric TNF-like molecule that is expressed mainly on activated Th cells [198]. Ligation of CD40 on DC induces maturation, reflected in production of high levels of IL-12, enhanced T cell stimulatory capacity and improved DC survival. Triggering CD40 on DC empowers these cells to activate naïve CD8+ CTL [9, 10, 13, 195, 199, 200].

Such conditioning or licensing of DC can be achieved by CD154-expressing Th cells, by soluble CD40L trimers or CD40 monoclonal Ab [197]. APC activation through CD40-CD40L interactions represents a crucial immunoregulatory step for the establishment of protective T cell immunity against pathogens and tumors [195]. M-DC are known to mature into the most potent human Th1-type-inducing APC upon CD40 ligation [8, 21]. It has been shown that CD40L has different effects on the different blood DC subsets. It appears that P-DC activated by CD40L induce Th2 differentiation, whereas M-DC activated by CD40L induce Th1 differentiation [21, 39]. CD40 ligation bypasses the need for CD4+ T cell help [13], although CD40 ligation cannot replace CD4+ T cell help for CD8+ CTL responses under some conditions [201].

Ag-Antibody Complexes and Fc Receptors

In addition to microbial and inflammatory products as well as CD4+ Th-dependent CD40 ligation, Ag-Ab IC are able to induce DC maturation in vitro [139, 148]. This

enables DC to prime peptide-specific CD8⁺ CTL in vivo, independently of CD4⁺ Th cells [148, 157].

DC express several receptors that facilitate the internalization and presentation of Ag, including C-type lectin receptors such as the mannose receptor and DEC205, as well as receptors for the Fc domain of Ig (FcγR and FcεR, which bind the Fc domain of IgG and IgE, respectively) [84, 85, 139, 148]. Three classes of FcγR have been recognized to date: FcγRI, FcγRII and FcγRIII in humans. In mouse, in addition to these three classes of FcγR, a novel FcγR has been discovered recently: FcγRIV [202]. Each class of FcγR consists of several individual receptor isoforms [203]. FcγRI is a high-affinity receptor capable of binding monomeric IgG. FcγRII and FcγRIII are low-affinity receptors, capable of binding only multimeric IgG. FcγRIV binds IgG2a with an intermediate affinity (40-fold higher than FcγRIII) [202]. They are structurally related receptors, with either two (FcγRII, FcγRIII and FcγRIV) or three (FcγRI) Ig-like extracellular domains, a single transmembrane domain and cytoplasmic regions of variable length. The first extracellular Ig-like domain and part of the second one of FcγRI are highly homologous with the corresponding domains of the low-affinity receptors, while the third Ig-like extracellular domain accounts for the unique ability of this receptor to bind monomeric IgG [204]. Functionally FcγR can be divided in two groups: activating FcγR (human FcγRIa, FcγRIIa and FcγRIIIa, murine FcγRI, FcγRIII and FcγRIV) and inhibiting FcγR (human FcγRIIb1 and FcγRIIb2, murine FcγRIIb1 and FcγRIIb2). With the exception of FcγRIIa, the activating receptors are associated with various subunits (γ-, ζ- and β-chains) that contain one or more cytoplasmic immunoreceptor tyrosine-based activation motifs (ITAM), which are conserved among a number of activating receptors, like the B cell and T cell receptors. The single chain FcγRIIa contains a single ITAM motif in its cytoplasmic tail. The inhibiting receptors, in contrast, contain an immunoreceptor tyrosine-based inhibition motif (ITIM) in their intracellular region. Human blood DC express several types of FcγR, including type I (FcγRI, CD64) and type II (FcγRII, CD32) [30]. Murine spleen- or bone-marrow-derived DC express all four FcγR [FcγRI, FcγRII, FcγRIII (CD16) and FcγRIV] [139, 202].

FcγR bind IC with very high affinity and may be functionally considered as Ag receptors. Targeting Ag to FcγR promotes cross-presentation by several orders of magnitude in DC. The FcγR-mediated cross-priming pathway requires the TAP1-TAP2 transporter and is sensitive to

lactacystin, a proteasome inhibitor, indicating that after internalization, IgG-complexed Ag are transferred into the cytosol and reach the conventional MHC class I Ag presentation pathway [205, 206]. FcγR engagement induces full DC maturation, reflected by increased levels of MHC and costimulatory molecules (CD40, CD80, and CD86) at the cell surface and production of high levels of IL-12. FcγR-mediated internalization of IC by DC fulfills a dual role in T cell priming to exogenous Ag: enhancement of Ag uptake by DC and activation of DC, resulting in 'licence to kill' [148, 157]. Simultaneous induction of maturation and MHC class I- and class II-restricted presentation by a single receptor-ligand interaction should result in efficient T cell priming in vivo [139]. Thus, the presence of specific Ab can crucially affect the induction of cellular responses.

DC Migration

Upon activation, DC travel to the lymphoid organs such as the spleen and the lymph nodes. There, DC attract T and B cells by releasing chemokines and maintain the viability of recirculating T lymphocytes [5, 161, 174]. Migration of DC is tightly regulated as a function of maturation [207, 208]. A variety of cytokines and chemokines [e.g. GM-CSF, TNF-α, IL-1, macrophage inflammatory protein (MIP)-1α and β], but also nonchemokine chemotactic agonists, lipid mediators and membrane proteins modulate DC movement and maturation [209]. The specific effects of chemokines on target cells are mediated through specific serpentine receptors that belong to the seven transmembrane, G protein-coupled receptor (GPCR) superfamily, but have unique structural features different from other GPCR [210].

In response to the appropriate stimuli DC upregulate CC chemokine receptor-7 (CCR7; also designated as EBI1), a receptor that drives DC migration to the lymphoid vessels and T cell areas of secondary lymphoid organs [37, 174, 210, 211]. In the T cell areas DC produce chemokines such as DC-CK-1 and MDC, which chemottract naïve and memory T cells, respectively [212–214]. In response to LPS, M-DC upregulate CCR7 and downregulate CCR5 expression. Upon virus infection or exposure to bacterial DNA (CpG), P-DC upregulate CCR7 and downregulate CXCR3. Both DC subsets respond to CD40L by upregulation of CCR7. Switch in chemokine receptor expression is accompanied by upregulation of B7.1, B7.2, CD83 and HLA-DR. Interestingly, the extent of CCR7 upregulation is consistently

much higher in P-DC than in M-DC [17, 37]. CCR7 ligands 6Ckine and MIP-3 β are extremely potent inducers of MHC class II^{high} B7-2^{high} DC migration [215]. 6Ckine is also a ligand for CXCR3 [215].

Immature and mature DC are not recruited by the same chemokines. Immature DC respond to many chemokines (MIP-1 α , MIP-1 β , MIP-5, MCP-3, MCP-4, RANTES, TECK, and SDF-1) and in particular to MIP-3 α , which acts through CCR6, a receptor mainly expressed in DC and lymphocytes. In contrast, mature DC have lost their responsiveness to most of these chemokines through receptor downregulation or desensitization, but acquire responsiveness to MIP-3 β and 6Ckine as a consequence of CCR7 upregulation. MIP-3 α mRNA is only detected within inflamed epithelial crypts of tonsils, the site of Ag entry known to be infiltrated by immature DC, whereas MIP-3 β and 6Ckine are specifically expressed in the T-cell-rich areas where mature DC home [216, 217].

Control of the Type of T Cell Response by DC

Upon the encounter of an infectious organism it is important that an immune response is initiated, but it is equally important that the response is appropriate. On the other hand no immune response should be initiated to self-Ag. DC are responsible for priming effector and memory T cells as well as for the induction of central and peripheral tolerance. It is essential that DC alert the T cells to the presence of infection and provide them with information about the nature of the infectious organism. Much of this information is transmitted as signal 3 cytokines and other molecules that skew emerging T cell responses. DC do not have the exquisite Ag discrimination ability conferred by clonally distributed receptors and can only act as advisors, providing T cells with information. T cells are the ultimate decision makers. Cytokine production by DC is subject to a tight regulation, which is particularly relevant in the case of IL-12, the prototypic Th1-polarizing cytokine. IL-12 production is elicited by most pathogens and is potently boosted by activated Th cells through CD40L [218]. However, IL-12 is not induced by some maturation stimuli, such as TNF- α , IL-1, cholera toxin or FasL. IL-12 production can be modulated by cytokines and mediators present during induction of maturation [18]. Thus, IFN- γ and even IL-4 enhance IL-12 production induced by appropriate stimuli, while PGE₂ and IL-10 exert an inhibitory effect. Moreover, IL-12 production by DC is restricted

to a narrow temporal window after induction of maturation [83]. The capacity to induce Th2 responses is a property of DC that do not produce Th1-polarizing cytokines.

In addition to the importance of inducing the appropriate type of response, it is as important not to induce a response to self-Ag. The role of DC in the maintenance of peripheral tolerance is becoming clear. DC maturation is recognized as a key event in the induction of the immune response [4]. DC maturation is associated with a rapid relocalization of Ag-bearing DC to the T cell zone of secondary lymphoid organs. Two models have been proposed for self/nonself discrimination: the 'pattern recognition model' [219] and the 'danger model' [220]. The first model suggests that DC possess pattern-recognition receptors that recognize conserved motifs on invading pathogens which serve as cues to upregulate stimulatory function. Strong evidence for this model came from the discovery of TLR in mammalian cells [155]. The latter model proposes that parasites that do not pose a threat to the host need not elicit immune responses, and only those causing damage require a response. To prevent autoimmunity, DC should induce peripheral tolerance to dying noninfected (or infected by harmless agents) tissues and immunity to harmful infectious agents. Therefore, the release of self-molecules, such as heat shock proteins or mitochondrial products, would serve as 'danger signals' to instruct DC to upregulate stimulatory function. Support for this model comes from the finding that necrotic cells release heat shock proteins that are capable of activating DC [221]. Immature DC are very efficient in the uptake of apoptotic cells and the presentation of self-Ag to T cells [22, 222, 223]. DC that have captured apoptotic cells do not mature and induce tolerance [224, 225]. Thus, in contrast to induction of T cell responses by mature DC, immature DC are involved in the maintenance of tolerance to self-Ag by defining self in the periphery. Furthermore, immature DC are continuously produced from the bone marrow and are widely distributed in the body, giving these cells the opportunity to constantly sample different self-Ag and present them to T cells in the draining lymph node. This presentation of tissue-specific self-Ag under noninflammatory conditions can occur without full deletion of autoreactive T cells or induction of active autoimmunity [226]. T cell recognition of self-peptide/MHC complexes on non-fully mature DC can induce tolerance by deletion of specific T cells [22, 85, 135, 222]. Recent studies have indeed shown the capacity of immature DC to induce peripheral tolerance to self-Ag by promoting naïve CD4⁺ and CD8⁺ cells to differ-

entiate into IL-10-producing T regulatory cells [227, 228]. These cells inhibit the mature DC-mediated Th1 T cell differentiation and suppress CD8+ memory T cell responses [227, 228].

The communication between DC and T cells is a dialogue rather than a monologue. T cells respond to DC and vice versa. Cells of the immune system can produce a positive feedback mechanism that increases the production of IL-12 by DC [229]: IL-12 stimulates directly NK cells and skews the Th response towards a Th1 response, leading to IFN- γ production, which augments the synthesis of IL-12 by DC. On the other hand, CD40L expression on activated Th cells triggers the CD40 molecule on DC, thereby inducing the production of high levels of IL-12.

Thus, the Th-polarizing capacity of DC is contingent on a number of variables that includes the microenvironment, the maturation stimuli and the kinetics of maturation.

DC and Cancer

Neoplastic disease represents the escape of cellular growth from normal host regulatory mechanisms. Successful tumors are able to escape the immune system by several mechanisms. Tolerance occurs in particular because the majority of the tumors, like most normal tissue, lacks costimulatory properties themselves and does not release danger signals. Tumor cells fail to activate the DC to an immunizing state and shed tumor Ag or apoptotic tumor cells are cross-presented by DC in a tolerizing state. By masquerading as normal tissues, tumors can cause tolerance upon cross-presentation of tumor Ag [230–232]. Thus, tumors may tolerize reactive T cells in the same way that self-tissues maintain tolerance against autoimmunity. Recently, it has been shown in a mouse model that in tumor draining lymph nodes tumor Ag is cross-presented by immature DC rather than directly by tumor cells. This presentation by immature DC did not induce CTL with efficient effector function [233]. Importantly, presentation of Ag by immature DC did not induce deletion Ag-specific CD8+ T cell tolerance, T cell anergy or T cell ignorance. Rather, a central memory type of CD8+ T cells was induced that could be rescued upon encounter of Ag presented by mature DC [234]. Tumors typically dampen the expression of MHC class I, the Ag-presenting molecules for CD8+ CTL and valuable tumor Ag may not be processed and presented. Several tumors exhibit deficiencies in genes involved in Ag processing or suppress

DC function. Moreover, tumors may block the development of immunity more globally by inhibiting the differentiation or maturation of DC. Tumors can induce apoptosis in DC, so that they are unavailable to generate a productive immune response [235–237]. Furthermore, tumors develop antiapoptotic mechanisms, where apoptosis is the basis for tumor killing by immune cells and provides a way for DC to capture and process Ag from tumors [238, 239]. All these possibilities are based on inefficient Ag presentation and T cell priming.

Early data suggestive of a role of DC in tumor defense were based on studies in experimental animals, or on a quantification of DC in the tumor areas and comparison to the clinical outcome of the patient. Delay in tumor growth or tumor regression was obtained when normal syngeneic DC were given to mice with palpable tumors [240]. Furthermore, irradiated tumor cells, retrovirally transfected with GM-CSF, induced specific and long-lasting tumor immunity. GM-CSF induced the influx of DC to the vaccination site, as well as their activation [241]. Effectiveness of DC vaccination in the induction of an effector T cell response able to protect against tumor growth was demonstrated in several mouse models [242–247]. Even in the case of already established tumors DC vaccination was of therapeutic benefit [247–249]. Although many mice were protected from or cured of tumors, these tumors are model systems, which are often highly immunogenic or have been manipulated to express foreign Ag to induce an Ag-specific response. Still, these observations have established the rationale for evaluating tumor Ag-bearing DC as therapeutic vaccines in humans.

Early immunohistochemical studies in humans on the presence of DC used CD1a expression to identify Langerhans cells (LC). A higher incidence of skin cancer was shown to be associated with a reduced number of LC. Several studies on human tumors have suggested that patients with high numbers of DC present in the tumors survived longer than patients with few or no DC in the tumor [250].

There is considerable interest in the possibility to use DC for the induction of antitumor responses. DC act as nature's adjuvants for regulating Ag-specific immunity. DC are both efficient and specialized in Ag presentation and T cell priming. In addition to T cells, DC can mobilize and activate NK and NKT cells, which can eliminate targets that dampen presentation on MHC class I to escape CTL recognition. Activating host DC in situ by intratumoral or systemic administration of DC-mobilizing and DC-activating cytokines is a way to circumvent im-

mune escape. A more interesting approach to exploit DC function in the setting of human cancer is the generation of DC from a patient ex vivo, charging the cells with tumor Ag and then readministering them to enhance cell-mediated resistance. Advantages of the use of DC over direct immunostimulation of patients are that the access of tumor Ag to the DC is ensured and that the quality of the DC can be manipulated.

Induction of CD8⁺ CTL, which kill virus-infected as well as malignant cells, is the goal of many vaccine strategies to treat cancer. CD8⁺ T cells have been shown to recognize processed and presented MHC class I binding epitopes from many different tumor Ag. Immunization with Ag-pulsed or transfected DC efficiently primes CD8⁺ T cells, resulting in protective immunity against infectious viruses and tumors. Crucial tumor-specific CD4⁺ help for CTL-mediated tumor protection was documented in different tumor models in mice [251]. Although cytokines secreted by CD4⁺ cells may have some role, recent evidence indicates that the major mechanism of CD4⁺ help for CTL is of a cell-cell cognate nature. CD40L which becomes upregulated on CD4⁺ cells during activation triggers CD40 on DC, thereby empowering those cells to prime CTL [10, 13, 200]. Furthermore, CD4⁺ cells are thought to be important in providing memory responses and extending the CD8⁺ response. Thus for intracellular tumor-associated Ag, CD8⁺ CTL may be the primary immune effector cell, but the activity and longevity of this response will likely be enhanced by CD4⁺ Th1 cell activation. For cell surface Ag, both CTL and humoral responses may mediate antitumor activity, again attesting to the importance of activating CD8⁺ and CD4⁺ T cells.

Clinical Trials with DC

With the recent advance in our knowledge of the differentiation and growth of DC, it is now possible to grow DC in sufficient numbers for vaccine production. DC for clinical use can be grown in culture from progenitors contained within bone marrow or blood. DC generated from human CD34⁺ precursors differentiate in the presence of GM-CSF plus TNF- α along two distinct pathways [252, 253]. CD1a⁺CD14⁻ intermediates give rise to CD1a⁺CD14⁻ cells resembling LC, and CD1a⁻CD14⁺ intermediates give rise to CD1a⁻CD14⁺ cells that have thus been termed M-DC. A more convenient source of cells are DC derived from monocytes. Human CD14⁺ monocytes differentiate into cells resembling immature DC upon culture in GM-CSF plus IL-4 (or IL-13). These immature DC mature when exposed to microbial products (e.g. LPS), cytokines (e.g. TNF- α) or by CD40 ligation and are applicable in the clinic [254–257].

DC-based immunotherapeutic approaches have now reached the stage of human clinical trials. Initial results have been reported [reviewed and discussed in 258]. These studies revealed that DC-based vaccines can be applied safely without significant side effects. Clinical remissions were detected only in a subpopulation of patients. It is likely that clinical DC therapy can only reach large amounts of patients if activated DC can be obtained by simple procedures with standardized conditions as a cellular blood transfusion product. The efficacy of therapy with ex vivo activated blood-derived DC will have to be compared with that of directly in vivo activated DC.

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