Summary. Dendritic cells (DCs) are the most potent antigen-presenting cells, and are regarded as “natural adjuvants” for the induction of primary T or T-dependent immunity. DCs in the peripheral sites capture and process antigens. Encounter of exogenous or endogenous stimuli mature the function of DCs, and they thus acquire T-cell stimulatory capacity and distinct chemotactic behavior which enables them to migrate to lymphoid tissue. In the secondary lymphoid organs, they present antigens to T- and B-cells and stimulate their proliferation. Dendritic cells are also involved in tolerance induction, in particular, to self antigens. DCs also play a key role in the transmission of many pathogens, and therefore may become targets for designing new therapies. DCs have been manipulated in vitro and in vivo for cancer immunotherapy. In this article, we provide a concise overview of DC biology and its current and future role in clinical settings.

Key words: Dendritic cells, Immunity, Chemokine, Maturation, Cancer immunotherapy

Introduction

Antigens need to be “processed” and “presented” by antigen-presenting cells (APCs) before they can be recognized by specific effectors. Any cells expressing major histocompatibility (MHC) or related molecules (e.g., CD1) which carry antigenic elements (e.g., peptides) that may be recognized by lymphocytes can be regarded as antigen-presenting cells (APCs). Dendritic cells (DCs, Fig. 1) are professional APCs and are more potent than others (macrophages, B-cells, etc) in that DCs possess the strongest T-cell stimulatory capacity. It is estimated that one DC can turn on 100-3,000 T-cells. There is also evidence that DCs can activate B- and NK cells (Banchereau and Steinman, 1998).

Dendritic cells were first described by Steinman and Cohn in 1973 as a trace leukocyte population isolated from mouse lymphoid tissue with the properties of low buoyant density, transient adherence to tissue culture plastic and irregular shape (Steinman and Cohn, 1973). It is now clear that DCs should be regarded as a family that includes various types of cells at different locations, yet with one common role, i.e., initiation of immunity. The members of this family are widely resident in virtually all tissues of the body, such as skin epidermal Langerhans cells (LCs), dermal (interstitial) DCs, splenic marginal DCs, interdigitating DCs in T-cell zones of the secondary lymphoid tissues, germinal center DCs, thymic DCs, and peripheral blood DCs, etc. The study of DCs in the past, however, was restricted by their rarity in peripheral blood or tissues until methods for in vitro culture in a large quantity were available. DCs can now be cultured in vitro from CD14+ monocytes (Sallusto and Lanzavecchia, 1994) and CD34+ stem cells (Caux et al., 1992) in the peripheral blood or CD4+CD3-CD11c+ plasmacytoid cells (O’Doherty et al., 1994; Grouard et al., 1997; Olweus et al., 1997) in humans, or from bone marrow cells in mice. DCs have attracted much attention from clinicians as DCs potentially can be and indeed have been employed for the treatment of infectious diseases or cancers due to their unique characteristics in initiating naïve T-cells.

Differentiation and development of DCs

Multiple distinct lineages and differentiation pathways of DCs exist (Fig. 2). At least two DC precursors are present in humans. Myeloid DCs derive from peripheral blood monocytes in culture with GM-CSF and interleukin (IL)-4 (Romani et al., 1994) or after phagocytosis and transendothelial migration (Randolph et al., 1998). The other type is lymphoid DCs that derive from plasmacytoid cells in blood or tonsils after culture with IL-3 (O’Doherty et al., 1994; Grouard et al., 1997; Olweus et al., 1997). DCs in peripheral tissues are in an “immature” state because they do not yet express abundant co-stimulatory molecules such as CD80 (B7.1), CD86 (B7.2), CD54 (ICAM-1) etc. at the cell surface that are essential for T-cell stimulation. Nevertheless, immature myeloid DCs are capable of taking up antigens (phagocytosis or
pinocytosis) in the periphery (skin or mucosa). After picking up the antigens myeloid DCs subsequently migrate in response to various chemoattractive signals (see below) via lymph to the draining lymph nodes or via blood to the spleen. When they arrive at the T-cell-rich region in the lymph nodes or spleen, DCs become "mature" and ready to "wake up" naïve T-cells for primary T-cell immunity or to "fuel up" the memory T-cells for secondary T-cell responses. Maturation of migrating DCs is accompanied by the disappearance of antigen-capturing apparatus and by the upregulation of essential co-stimulatory molecules. In contrast, immature lymphoid DCs have little capacity to phagocytose or pinocytose antigens (Grouard et al., 1997). However, both mature myeloid and lymphoid DCs induce strong proliferation of allogeneic naïve CD4+ T-cells.

Multiple endogenous or exogenous stimuli can drive immature DCs into cells with a mature, costimulatory phenotype. These stimuli include the CD40 ligand (mainly expressed on activated CD4+ T-cells), cytokines such as GM-CSF, TNF-α and IL-1, prostaglandin E, bacterial lipopolysaccharide (LPS) (Sallusto et al., 1995), whole bacteria (Winzler et al., 1997), viral double-strand RNAs (Cella et al., 1999), CpG oligonucleotides (Verdijk et al., 1999), or monocyte-conditioned medium (Sallusto and Lanzavecchia, 1994; Bender et al., 1996; Romani et al., 1996; Cella et al., 1997). In contrast, IL-10 (Allavena et al., 1998), TGFβ (Ogata et al., 1999) and vascular endothelial growth factor (VEGF) (Oyama et al., 1998) inhibit this maturation process. In response to activating stimuli, DCs express high levels of the NF-κB family of transcriptional control proteins for the synthesis of immune and inflammatory proteins (Granelli-Piperno et al., 1995). Moreover, upon maturation DCs begin to load newly synthesized MHC molecules (e.g., MHC class II) with antigenic peptides that derive from phagocytosed or pinocytosed and processed antigens, and display these complexes on the cell surface. DCs also begin to express co-stimulatory molecules required for T-cell activation, as well as CD83, a maturation marker for human DCs (Zhou et al., 1995).

Migration of DCs

DCs are highly mobile. In the absence of pathogens, a certain proportion of DCs seem to migrate in a steady state for the replenishment of the immature DCs in the periphery, such as LCs in skin and resident DCs in dermis or solid organs such as heart, kidney, and liver. Encounter of pathogens and antigens also mobilizes DCs. For example, DCs migrate following the application of fluorescent allergens on skin, and fluorescent DCs can be identified in the draining lymph nodes 24 hours afterwards. DCs mobilize into the airway epithelium after inhalation of viruses or bacteria (McWilliam et al., 1996). After heart and skin allograft

**Fig 1.** DCs under a light microscope. Microscopic features of day-7 monocyte-derived cytokine (GM-CSF/IL-4)-cultured DC clusters where dendritic projection (empty arrow) or veils (black arrows) are characteristic. x 200
transplantation, DCs migrate out from the grafts to the lymphoid tissues. After reaching secondary lymphoid tissues, DCs presumably die within them because DCs are always present in the afferent lymph, but have never been found in the efferent lymph (Fig. 3).

Chemotactic cytokines (chemokines) have chemotactic activities for leukocytes, including DCs. Human chemokines can be classified according to their cysteine motifs close to the N terminus: CC and CXC chemokines, in which the first two cysteines are adjacent or separated by a single residue, respectively. Two minor categories, C and CX3C chemokines, also play a role in leukocyte migration (Mackay, 2001). Chemokines bind on chemokine receptors, members of the G-protein-coupled superfamily. Immature monocyte-derived DCs respond potently to the CC chemokines MIP-1α, MIP-1ß, RANTES, MCP-3 and weakly to MIP-3ß and CXC chemokine SDF-1α (Lin et al., 1998). Most of these chemokines are "inducible" at the site of inflammation. After DC maturation, the responsiveness to CC chemokines is reduced or abolished, but the responsiveness to MIP-3ß and SDF-1α, which are constitutively expressed in the central lymphoid tissues, is dramatically increased. These changes in the responsiveness to chemokines correlate with the changes of the expression of the chemokine receptors on DCs: after maturation the surface expression of CC chemokine receptor 5 (CCR5, receptor for MIP-1α, MIP-1ß and RANTES) is reduced while that of CXCR4 (receptor for SDF-1α) and CCR7 (receptor for MIP-3ß) is enhanced (Lin et al., 1998). Therefore, there are two sets of chemokines that regulate DC chemotaxis and migration: one set of "inducible" chemokines attract DCs towards the site of inflammation or infection and the other set of "constitutive" chemokines are responsible for allowing DCs to go to the secondary lymphoid tissues and initiate T-cell responses after local activation/maturation. Maturation of DCs by CD40 ligation also increases the surface expression of CX3C of DCs, which may enhance the conjugate formation of DCs and T-cells (Papadopoulos et al., 1999).

Regulation of T- and B-cell immunity by DCs

Antigens that are not "handled" by DCs will be ignored by T-cells, whereas those "manipulated" and "presented" by DCs can stimulate naïve T-cells that subsequently undergo proliferation and differentiation, and in special conditions, deletion. Myeloid DCs, after maturation, secrete IL-12 (an important Th1 cytokine) and, along with the increased expression of costimulatory molecules, drive clonal expansion of committed T-cells. There is a sophisticated cross-talk between DCs and T-cells. Activated T-cells can also deliver signals such as the CD40 ligand (gp39) to CD40 of DCs, thereby enhancing their T-cell stimulatory capacity, prolonging their survival, and further increasing IL-12 production (Caux et al., 1994). The same outcome can be triggered through the interaction between the TNF-superfamily (e.g., TRANCE) on activated T-cells and receptors on DCs (Wong et al., 1997). In contrast, mature lymphoid DCs produce type 1-interferon (IFN-α and β) rather than IL-12. As a result, myeloid DCs induce Th1 T-cell responses, while lymphoid DCs probably induce Th2 T-cell responses. Moreover, DCs may be involved in the induction of T-cell tolerance. DCs in the thymus generate (central) tolerance by deleting self-reactive T-cells (Brocker et al., 1997). DCs in the peripheral lymphoid tissues can also
induce peripheral T-cell tolerance to self antigens (Inaba et al., 1998). There is new evidence that DCs may induce expansion and differentiation of regulatory or suppressor T-cells (Roncarolo et al., 2001). For example, immature DCs prime regulatory T-cells for the production of IL-10 (Dhodapkar et al., 2001). Therefore, the influence on T-cell activities by DCs is heterogeneous and regulated in a sophisticated manner.

DCs may also stimulate B-cell immunity because DCs are present in the germinal centers, and signals from DCs (i.e., OX40L) drive T-helper cell migration into B-cell areas. The type 1 IFN produced by lymphoid DCs also enhances antibody responses and isotype switching in vivo.

DCs in clinical settings

DCs in HIV infection

Another reason DCs attract attention in recent biomedical research is because DCs play an essential role in the setting of HIV infection. In an animal study, DCs were found to be the first cells to be targeted by Simian Immunodeficiency Virus (SIV-1) (Spira et al., 1996). HIV-1 binds on its primary receptor CD4 and coreceptor CCR5 [for monocyte (M)-tropic HIV-1] or CXCR4 [for T lymphocyte (T)-tropic HIV] for cell entry. DCs express all these molecules (Lin et al., 1998; Sewell and Price, 2001). Both the M- and T-tropic HIV-1, HIV-2 as well as SIV can bind through its envelope protein (i.e., gp120) in an infectious form to dendritic cell-specific ICAM-3 grabbing non-integrin (DC-SIGN), a type-II membrane protein C-type lectin domain specifically expressed on dendritic cells (Geijtenbeek et al., 2000a,b). HIV is subsequently attached on the cell surface or internalized into DCs in a non-lysosomal compartment to prevent degradation. After DCs migrate to T-cell areas in the secondary lymphoid tissues, viruses are delivered to T cells and explosive replication ensues. DCs thus are regarded as a “Trojan Horse” in HIV infection. M-tropic, but not T-tropic, HIV-1 were found to attract immature myeloid DCs to the site of infection by signaling through the viral envelope protein gp120 binding onto CCR5 (Lin et al., 2000). By recruiting the immature DCs and subsequent binding on DC-SIGN, M-tropic HIV-1, which is responsible for primary infection, may thus exploit the migration of DCs as they mature to gain access to lymphoid tissues, thereby increasing the infectivity as well as productivity when DCs fuse with T-cells in the lymphoid tissues for explosive viral production.

Other infectious diseases

As a sentinel, DCs usually encounter pathogens before other cells. Their function, therefore, is influenced in a way that downstream T-cell function in adaptive immunity is modulated accordingly. For example, measles infection induces phenotypic and functional maturation of DCs (Schnorr et al., 1997), blocks their allo-stimulatory properties of CD4+ T-cells (Grosjean et al., 1997) and regulates their survival (Fugier-Vivier et al., 1997), thereby resulting in immunosuppression.

Immature DCs also engulf the parasitic organism by macropinocytosis. LCs can be infected by Leishmania in dermal infiltrates of skin lesion (Moll, 1993), through binding DC-SIGN (Colmenares et al., 2002). Infected DCs migrate to lymph nodes where they become mature and IL-12 p70 is produced from infected DCs to activate Leishmania-specific T-cells (Marovich et al., 2000). Another parasite, Toxoplasma gondii, also induces production of IL-12 by dendritic cells in a CD40 ligand-independent manner and redistributes DCs in T-cell areas (Reis et al., 1997). Subversion of DC function is also observed in the setting of Plasmodium falciparum infection. DCs bind and internalise Plasmodium falciparum-infected red blood, and the adherence of Plasmodium-infected erythrocytes on DCs, via receptors CD36 and CD54, leads to inhibition of DC maturation and consequent poor T-cell stimulatory capacity (Urban et al., 1999). Furthermore, the bacterium C. pneumoniae stimulates bone marrow-derived DCs to secrete proinflammatory cytokines and up-regulates the surface expression of MHC class II molecules, CD40, CD80 and CD86 on BMDC (Prebeck et al., 2001).

Apart from HIV, cytomegalovirus (CMV) is another example of a virus remaining latent in DCs (Hahn et al., 1998). CMV induces lytic infection of DCs in vitro (Riegler et al., 2000). CMV expresses glycoprotein UL18, an MHC class I homologue and also decays of inhibitory leukocyte immunoglobulin (Ig)-like receptor 1 (LIR-1) that is expressed on DCs. Binding of UL18 to LIR-1 inhibits DC differentiation and release of cytokines, deregulates viral response and suppresses the negative regulatory function of LIR, resulting in CMV replication (Cosman et al., 1997, 1999). Recent studies have also demonstrated that CMV envelope protein, glycoprotein B, binds on DC-SIGN and DC-SIGNR for the transmission of CMV to permissive cells, leading to productive infection in trans (Halary et al., 2002).

Interestingly, immature DCs also capture and internalize Candida albicans after ligation with DC-SIGN, targeting to DC-SIGN-enriched vesicles (Cambi et al., 2003). The downstream response, however, depends on in which form the Candida is present. The yeast form of Candida albicans activates DCs that in turn induce Th1 immunity, whereas the Th2 response is triggered if DCs are activated by the hyphal forms (Fe´ d’Ostiani et al., 2000; Romani et al., 2002).

Human skin LCs are also targets of dengue virus and the infection is through the DC-SIGN as well (Wu et al., 2000; Tassaneetrithep, 2003). Dengue virus (DV)-stimulated DCs express maturation markers such as B7-1, B7-2, HLA-DR, CD11b, and CD83. Furthermore, the infection of DC by DV induces production of TNF-α and IFN-α but not IL-6 or IL-12 (Ho et al., 2001). Upon
the addition of IFN-α, there is an enhanced activation of dengue virus-infected DCs and an enhanced dengue virus-induced IL-12 p70 release (Libraty et al., 2001).

**DCs in transplantation**

The induction of allograft rejection can be mediated at least in two ways. First, direct sensitization: donor APCs that are carried over with the engraftment migrate out from the grafts to the recipient’s lymphoid tissues and trigger rejection. Second, indirect sensitization: the recipient’s APCs migrate into the graft tissues, pick up the transplantation antigens (mainly the donor’s MHC molecules), and migrate back to the recipient’s lymphoid organs and present them to its own T-cells to induce allograft rejection. It has been shown that in either case DCs are the most important APCs mediating the induction of transplantation rejection. In thyroid transplantation, the depletion of HLA-class II high cells may result in prolonged graft survival (Iwai et al., 1989). Furthermore, culture of mouse bone marrow cells with low-dose GM-CSF without IL-4 generates DCs with an immature phenotype that has little T-cell stimulatory capacity and does not mature in response to LPS. Intravenous injection of these cells of donor origin to allogeneic mice before transplantation results in indefinite survival of fully vascularized, heterotopic cardiac allografts (Lutz et al., 2000). Recently, there has been more and more interest in the study of the regulatory T-cell function modulated by DCs in allograft transplantation setting.

**DCs and cancers**

DCs also play a regulatory role in the immunity against malignancies. DCs are found in many tumor specimens, and statistically immature DCs (CD1a+) could be found in about 60% of tumor samples. The role of DCs in tumour immunity is multiple. Immature DCs can recognize tumor-associated or -specific molecules and thus present these antigens to T-cells. DCs may also activate NK or NK T-cells for direct or IFN-mediated killing of tumor cells. It has been shown that in patients with breast cancer the number of CD83(+) tumor-infiltrating DCs is inversely correlated with lymph node metastasis and with tissue expression of VEGF and TGF-β. There is also a significant association of an increasing number of CD83(+) intra-tumor DCs with a longer relapse-free time and overall survival. Furthermore, among patients with lymph node metastasis, the survival rate of those with a higher number of CD83(+) DCs is significantly better than that of patients with fewer (Iwamoto et al., 2003). However, studies from other tumors also suggest that local secretory proteins such as VEGF, IL-10 and IL-6 produced by tumor cells prevent DC maturation and function (Gabrilovich et al., 1996), which may result in a low allostimulatory capacity of DCs isolated from tumour samples. IL-10 can also inhibit the antigen-presenting function of DCs, thereby leading to induction of antigen-specific anergy that results in immunological tolerance to tumor cells (Enk et al., 1994, 1997; Steinbrink et al., 1999).

Because of the unique characteristics of DCs for the induction of primary T-cell immunity, DCs have been employed in vaccination for cancer immunotherapy in vivo. Earlier studies in mice showed that vaccination with bone marrow-derived DCs (BMDC) pulsed with antigens of C3 sarcoma and the 3LL lung carcinoma achieved 80% sustained tumor regression and tumor free status (Mayordomo et al., 1995). Immunization with BMDC fusions that express the MUC1 antigen resulted in the rejection of established metastatic tumors (Gong et al., 1998). Vaccination with BMDC pulsed with MCA-106 fibrosarcoma also inhibited the growth of

Table 1. Clinical trials of DC-based cancer immunotherapy

<table>
<thead>
<tr>
<th>MALIGNANCIES</th>
<th>ANTIGENS</th>
<th>DC SOURCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>B cell lymphoma</td>
<td>Ig idiotype</td>
<td>Peripheral blood DC</td>
</tr>
<tr>
<td>Multiple myeloma</td>
<td>Ig idiotype</td>
<td>Peripheral blood DC, BMDC</td>
</tr>
<tr>
<td>Melanoma</td>
<td>gp100 peptides, MART-1, MAGE-3, MAGE-1,</td>
<td>BMDC, MDDC</td>
</tr>
<tr>
<td></td>
<td>tyrosinase peptides, tumor lysate</td>
<td></td>
</tr>
<tr>
<td>Prostate cancer</td>
<td>PAP, PSMA</td>
<td>Peripheral blood DC, MDDC</td>
</tr>
<tr>
<td>Renal cell carcinoma</td>
<td>Tumor lysate</td>
<td>MDDC</td>
</tr>
<tr>
<td>Lung cancer</td>
<td>p53 and its mutants, ras peptides, tumor</td>
<td>MDDC</td>
</tr>
<tr>
<td></td>
<td>lysate</td>
<td></td>
</tr>
<tr>
<td>GI cancer</td>
<td>CEA CAP-1 peptide, CEA RNA, tumor lysate</td>
<td>MDDC</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>HER-2/neu RNA, CEA CAP-1 peptide, p53</td>
<td>MDDC</td>
</tr>
<tr>
<td></td>
<td>protein, tumor lysate</td>
<td></td>
</tr>
<tr>
<td>Pancreatic cancer</td>
<td>Tumor lysate</td>
<td>MDDC</td>
</tr>
<tr>
<td>Sarcoma</td>
<td>Tumor lysate</td>
<td>MDDC</td>
</tr>
<tr>
<td>Hepatoma</td>
<td>Tumor lysate</td>
<td>MDDC</td>
</tr>
<tr>
<td>Nasopharyngeal cancer</td>
<td>EBV-specific peptide</td>
<td>MDDC</td>
</tr>
<tr>
<td>Cervical cancer</td>
<td>Tumor lysate</td>
<td>MDDC</td>
</tr>
<tr>
<td>Neuroblastoma</td>
<td>Tumor lysate</td>
<td>MDDC</td>
</tr>
<tr>
<td>Ovarian cancer</td>
<td>Tumor lysate, total RNA</td>
<td>MDDC</td>
</tr>
</tbody>
</table>

Ig: Immunoglobulin; BMDC, CD34+ cell-derived DCs; MDDC: monocyte-derived DCs; PAP: prostatic alkaline phosphatase; PSMA: prostate-specific membrane antigen; CEA: carcinoembryonic antigen.
hepatic metastasis in B6 mice (DeMatos et al., 1998). Human trials in early years also had encouraging results. For example, immunization with DCs loaded with Epstein-Barr virus (EBV)-transformed B-lymphoblastoid cell lines improved the generation of EBV-specific cytotoxic T-cells for the treatment of post-transplantation lymphoma (Wheatley et al., 1998). Vaccination with autologous DCs pulsed with tumor-specific proteins in 4 patients with B cell lymphoma resulted in detectable anti-tumor immunity in all 4 patients in which 2 had complete tumor regression and 1 had a partial response (Hsu et al., 1996). Vaccination of malignant melanoma patients with peptide- or tumor lysate-pulsed DC resulted in 6/16 patients with a detectable clinical response rate and 5/16 with regression of metastasis in various organs and one additional minor response (Nestle et al., 1998). Recently, it has been shown that vaccination with DCs pulsed with HLA-restricted EBV-specific peptide epitopes also induced functional antigen-specific CTL immunity that might lead to partial tumor regression in patients with EBV-positive nasopharyngeal carcinoma (Lin et al., 2002). DC-based cancer immunotherapy conducted in human subjects in recent years also includes breast cancer, cervical cancer, prostate cancer, renal cell carcinoma, hepatocellular carcinoma, pancreatic cancer, colorectal cancer, lung cancer, leukemia and solid tumors in pediatric populations, etc (Table 1). However, the most optimal sources of tumor-associated antigens and the routes for delivering DCs need further investigation and experiences. Thus far, the preparation of the tumor-associated or tumor-specific antigens used for DC pulsing includes the whole tumor lysate, undefined acid-eluted peptides from autologous tumors, defined peptides with known sequences, antigen-carrying viral vectors for DC transfection, tumor-derived RNAs or DNAAs, exosomes derived from DCs, and fusion of DCs with tumor cells, etc. Routes of delivering DCs include intravenous, intranodal (direct injection into lymph nodes), intradermal, and subcutaneous injections. However, there is still no consensus as to which way of delivery may give the best immunological responses in human subjects. Furthermore, the numbers of DCs for injection may not correlate with the magnitude of desired antigen-specific responses. The vaccination schedule may also have an impact on the duration of the generated anti-tumor immunity, which is also an important issue that needs to be addressed.

Conclusion

In conclusion, the DC family is composed of various types of cells located all over the body with unique immunological characteristics for the initiation of immunity, in particular, primary T-cell responses. The significance of DCs to various diseases is being verified, and the potential clinical application for treatment of infectious diseases and malignancies is being examined. The convergence of information resulting from basic studies in DC immunobiology, along with the increasing sophistication in biotechnology has opened extraordinary possibilities for the development of effective vaccines in the context of DC immunotherapy. The study of dendritic cell biology has illustrated an excellent example of “from-bench-to-bedside” biomedical research.

References


Dendritic cells and their clinical application


Accepted September 29, 2002