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Review

Dendritic cells: sentinels against pathogens

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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 histocompactibility (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, liver 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.

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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), TGFß (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

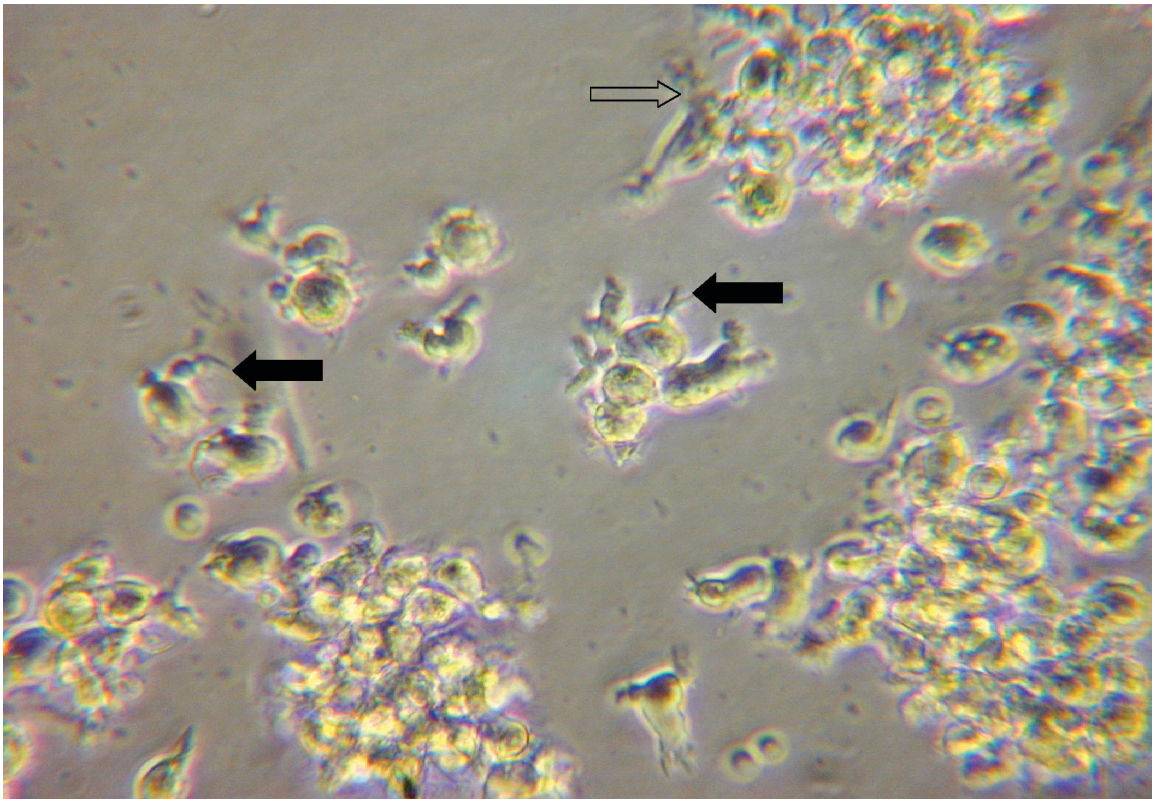

Fig 1. DCs under a light microscope. Microscopic features of day-7 monocyte-derived cytokine (GM-CSF/IL-4)-cultured DC clusters where dendritic projection (empty arrow) or veils (black arrows) are characteristic. x 200

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).

Chemoattractive 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:

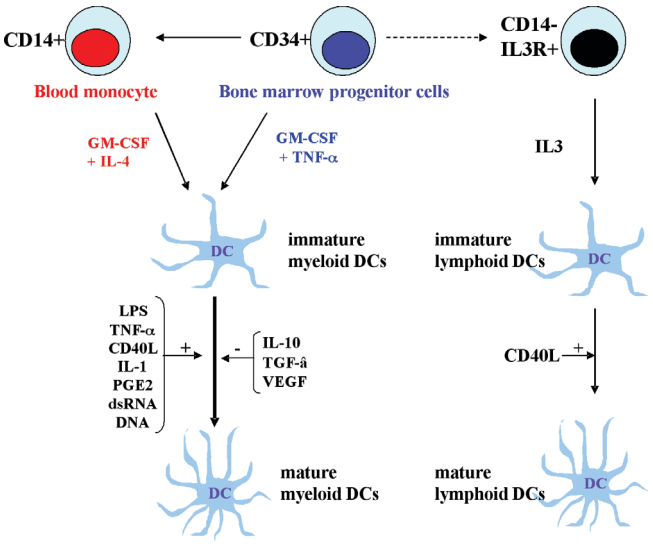

Fig 2. DC life cycle. Immature myeloid DCs can differentiate from CD34+ stem cells cultured with GM-CSF and TNF-α, or CD14+ monocytes cultured by GM-CSF and IL-4. Myeloid DCs can be matured by several exogenous and endogenous stimuli (see text). Immature lymphoid DCs can be derived from plasmacytoid cells (CD14-IL-3+) in blood or tonsils after culture with IL-3, and matured by CD40 ligand.

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 co-stimulatory 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 ß) 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

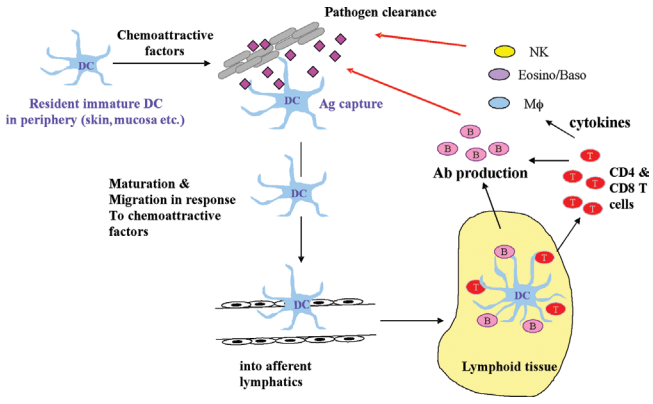

Fig 3. Migratory pathway of DCs. Immature myeloid DCs migrate to the site of inflammation in response to chemoattractive signals and phagocytose antigens in the periphery. They migrate to the secondary lymphoid tissues and become mature in presenting antigens and priming T- and B-immunity. They can also activate other immune effectors such as NK cells. These effectors in turn migrate to the site of microbial invasion to exercise their function of antigen clearance.

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 co-receptor 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.

Immatue 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 *Leishamania*-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 *Plasmondium falciparum* infection. DCs bind and internalise *Plasmondium falciparum*-infected red blood, and the adherence of Plasmondium-infected erythrocytes on DCs, via receptors CD36 and CD54, leads to inhibiton 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 BMDDC (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 decoy 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 *Candidia 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 over 90% of samples while mature DCs ($CD83^+$ $DC\text{-}LAMP^+$) could be identified 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-ß. 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

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

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 DNAs, 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

Allavena P., Piemonti L., Longoni D., Bernasconi S., Stoppacciaro A., Ruco L. and Mantovani A. (1998) IL-10 prevents the differentiation of monocytes to dendritic cells but promotes their maturation to macrophages. Eur. J. Immunol. 28, 359-369

Banchereau J. and Steinman R. (1998). Dendritic cells and control of immunity. Nature 392, 245-252.

Bender A., Sapp M., Schuler G., Steinman R. and Bhardwaj N. (1996). Improved methods for the generation of dendritic cells from nonproliferating progenitors in human blood. J. Immunol. Methods 196, 121-135.

Brocker T., Riedinger M. and Karjalainen K. (1997). Targeted expression of major histocompatibility complex (MHC) class II molecules demonstrates that dendritic cells can induce negative but not positive selection of thymocytes *in vivo*. J. Exp. Med. 185, 541-550.

Cambi A., Gijzen K., de Vries J.M., Torensma R., Joosten B., Adema G., Netea M., Kullberg B., Romani L. and Figdor C. (2003). The C-type lectin DC-SIGN (CD209) is an antigen-uptake receptor for Candida albicans on dendritic cells. Eur. J. Immunol. 33,532-528.

Caux C., Dezutter-Dambuyant C., Schmitt D. and Banchereau J. (1992). GM-CSF and TNF-alpha cooperate in the generation of dendritic Langerhans cells. Nature 360, 258-261.

Caux C., Vanbervliet B., Massacrier C., Azuma M., Okumura K., Lanier L. and Banchereau J. (1994). B70/B7-2 is identical to CD86 and is the major functional ligand for CD28 expressed on human dendritic cells. J. Exp. Med. 180, 1841-1847.

Cella M., Sallusto F. and Lanzavecchia A. (1997). Origin, maturation and antigen presenting function of dendritic cells. Curr. Opin. Immunol. 9, 10-16.

Cella M., Salio M., Sakakibara Y., Langen H., Julkunen I. and Lanzavecchia A. (1999). Maturation, activation, and protection of dendritic cells induced by double-stranded RNA. J. Exp. Med. 189, 821-829.

Cosman D., Fanger N. and Borges L. (1999). Human cytomegalovirus, MHC class I and inhibitory signalling receptors: more questions than answers. Immunol. Rev. 168, 177-185.

Cosman D., Fanger N., Borges L., Kubin M., Chin W., Patterson L. and Hsu M. (1997). A novel immunoglobulin superfamily receptor for cellular and viral MHC class I molecules. Immunity 7, 273-282.

Colmenares M., Puig-Kroger A., Pello O., Corbi A. and Rivas L. (2002). Dendritic cell (DC)-specific intercellular adhesion molecule 3 (ICAM-3)-grabbing non-integrin (DC-SIGN, CD209), a C-type surface lectin in human DCs, is a receptor for Leishmania amastigotes. J. Biol. Chem. 277, 36766-36769.

Dhodapkar M., Steinman R., Krasovsky J., Munz C. and Bhardwaj N. (2001). Antigen-specific inhibition of effector T cell function in humans after injection of immature dendritic cells. J. Exp. Med. 193, 233-238.

DeMatos P., Abdel-Wahab Z., Vervaert C. and Seigler H. (1998).

Vaccination with dendritic cells inhibits the growth of hepatic metastases in B6 mice. Cell. Immunol. 185, 65-74.

Enk A., Saloga J., Becker D., Mohamadzadeh M. and Knop J. (1994). Induction of hapten-specific tolerance by interleukin 10 in vivo. J. Exp. Med. 179, 1397-1402.

Enk A., Jonuleit H., Saloga J. and Knop J. (1997). Dendritic cells as mediators of tumor-induced tolerance in metastatic melanoma. Int. J. Cancer 73, 309-316.

Fe'd'Ostiani C., Del Sero G., Bacci A., Montagnoli C., Ricciardi-Castagnoli P., Spreca A. and Romani L. (2000). Dendritic cells discriminate between yeast and hyphae of the fungus *Candida albicans*: Implications for initiation of Th immunity in vitro and in vivo. J. Exp. Med. 191, 1661-1674.

Fugier-Vivier I., Servet-Delprat C., Rivailler P., Rissoan M., Liu Y. and Rabourdin-Combe C. (1997). Measles virus suppresses cell-mediated immunity by interfering with the survival and functions of dendritic and T cells. J. Exp. Med. 186, 813-823.

Gabrilovich D., Chen H., Girgis K., Cunningham H., Meny G., Nadaf S., Kavanaugh D. and Carbone D. (1996). Production of vascular endothelial growth factor by human tumors inhibits the functional maturation of dendritic cells. Nat. Med. 2, 1096-1103.

Geijtenbeek T., Torensma R., van Vliet S., van Duijnhoven G., Adema G., van Kooyk Y. and Figdor C. (2000a). Identification of DC-SIGN, a novel dendritic cell-specific ICAM-3 receptor that supports primary immune responses. Cell 100, 575-585.

Geijtenbeek T., Kwon D., Torensma R., van Vliet S., van Duijnhoven G., Middel J., Cornelissen I., Nottet H., KewalRamani V., Littman D., Figdor C. and van Kooyk Y. (2000b). DC-SIGN, a dendritic cell-specific HIV-1-binding protein that enhances trans-infection of T cells. Cell 100, 587-597.

Grouard G., Rissoan M., Filgueira L., Durand I., Banchereau J. and Liu Y. (1997). The enigmatic plasmacytoid T cells develop into dendritic cells with interleukin (IL)-3 and CD40-ligand. J. Exp. Med. 185, 1101-1111.

Grosjean I., Caux C., Bella C., Berger I., Wild F., Banchereau J. and Kaiserlian D. (1997). Measles virus infects human dendritic cells and blocks their allostimulatory properties for $CD4^+$ T cells. J. Exp. Med. 186, 801-812.

Gong J., Chen D., Kashiwaba M., Li Y., Chen L., Takeuchi H., Qu H., Rowse G., Gendler S. and Kufe D. (1998). Reversal of tolerance to human MUC1 antigen in MUC1 transgenic mice immunized with fusions of dendritic and carcinoma cells. Proc. Natl. Acad. Sci. USA 95, 6279-6283.

Granilli-Piperno A., Pope M., Inaba K. and Steinman R. (1995). Coexpression of NF-κB/Rel and Spl transcription factors in human immunodeficiency virus 1-induced, dendritic cell-T-cell syncytia. Proc. Natl. Acad. Sci. USA. 92, 10944-10948.

Halary F., Amara A., Lortat-Jacob H., Messerle M., Delaunay T., Houles C., Fieschi F., Arenzana-Seisdedos F., Moreau J. and Dechanet-Merville J. (2002). Human cytomegalovirus binding to DC-SIGN is required for dendritic cell infection and target cell trans-infection. Immunity 17, 653-664.

Hahn G., Jorges R., and Mocarski E. (1998). Cytomegalovirus remains latent in a common precursor of dendritic and myeloid cells. Proc. Natl. Acad. Sci. USA 95, 3937-3942.

Ho L., Wang J., Shaio M., Kao C., Chang D., Han S. and Lai J. (2001). Infection of human dendritic cells by dengue virus causes cell maturation and cytokine production. J. Immunol. 166, 1499-1506.

Hsu F., Benike C., Fagnoni F., Liles T., Czerwinski D., Taidi B., Engleman E. and Levy R. (1996). Vaccination of patients with B-cell lymphoma using autologous antigen-pulsed dendritic cells. Nat. Med. 2, 52-58.

Inaba K., Turley S., Yamaide F., Iyoda T., Mahnke K., Inaba M., Pack M., Subklewe M., Sauter B., Sheff D., Albert M., Bhardwaj N., Mellman I. and Steinman R. (1998). Efficient presentation of phagocytosed cellular fragments on the major histocompatibility complex class II products of dendritic cells. J. Exp. Med. 188, 2163-2173.

Iwai H., Kuma S., Inaba M., Good R., Yamashita T., Kumazawa T. and Ikehara S. (1989). Acceptance of murine thyroid allografts by pretreatment of anti-Ia antibody or anti-dendritic cell antibody in vitro. Transplantation 47, 45-49.

Iwamoto M., Shinohara H., Miyamoto A., Okuzawa M., Mabuchi H., Nohara T., Gon G., Toyoda M. and Tanigawa N. (2003) Prognostic value of tumor-infiltrating dendritic cells expressing CD83 in human breast carcinomas. Int. J. Cancer 104, 92-97.

Libraty D., Pichyangkul S., Ajariyakhajorn C., Endy T. and Ennis F. (2001). Human dendritic cells are activated by dengue virus infection: enhancement by gamma interferon and implications for disease pathogenesis. J. Virol. 75, 3501-3508.

Lin C., Suri R., Rahdon R., Austyn J. and Roake J. (1998). Dendritic cell chemotaxis and transendothelial migration are induced by distinct chemokines and are regulated on maturation. Eur. J. Immunol. 12, 4114-4122.

Lin C., Sewell A., Gao G., Whelan K., Phillips R. and Austyn J. (2000). Macrophage-tropic HIV induces and exploits dendritic cell chemotaxis. J. Exp. Med. 192, 587-594.

Lin C., Lo W., Lee T., Ren Y., Hwang S., Cheng Y., Chen C., Chang Y., Lee S., Rickinson A. and Tam P. (2002). Immunization with Epstein-Barr Virus (EBV) peptide-pulsed dendritic cells induces functional $CD8^+$ T-cell immunity and may lead to tumor regression in patients with EBV-positive nasopharyngeal carcinoma. Cancer Res. 62, 6952-6958.

Lutz M., Suri R., Niimi M., Ogilvie A., Kukutsch N., Rossner S., Schuler G. and Austyn J. (2000). Immature dendritic cells generated with low doses of GM-CSF in the absence of IL-4 are maturation resistant and prolong allograft survival in vivo. Eur J. Immunol. 30, 1813-1822.

McWilliam A., Napoli S., Marsh A., Pemper F., Nelson D., Pimm C., Stumbles P., Wells T. and Holt P. (1996). Dendritic cells are recruited into the airway epithelium during the inflammatory response to a broad spectrum of stimuli. J. Exp. Med. 184, 2429-2432.

Mackay C. (2001). Chemokines: immunology's high impact factors. Nat. Immunol. 2, 95-101.

Mayordomo J., Zorina T., Storkus W., Zitvogel L., Celluzzi C., Falo L., Melief C., Ildstad S., Kast W., Deleo A. and Lotze M. (1995). Bone marrow-derived dendritic cells pulsed with synthetic tumour peptides elicit protective and therapeutic antitumour immunity. Nat. Med. 1, 1297-1302.

Marovich M., McDowell M., Thomas E. and Nutman T. (2000). IL-12p70 production by Leishmania major-harboring human dendritic cells is a CD40/CD40 ligand-dependent process. J. Immunol. 164, 5858-5865.

Moll H. (1993). Epidermal Langerhans cells are critical for immunoregulation of cutaneous leishmaniasis. Immunol. Today 14, 383-387.

Nestle F., Alijagic S., Gilliet M., Sun Y., Grabbe S., Dummer R., Burg G. and Schadendorf D. (1998). Vaccination of melanoma patients with peptide- or tumor lysate-pulsed dendritic cells. Nat. Med. 4, 328-332.

Olweus J., BitMansour A., Warnke R., Thompson P., Carballido J., Picker L. and Lund-Johansen F. (1997). Dendritic cell ontogeny: a human dendritic cell lineage of myeloid origin. Proc. Natl. Acad. Sci. USA. 94, 12551-12556.

Ogata M., Zhang Y., Wang Y., Itakura M., Zhang Y., Harada A., Hashimoto S. and Matsushima K. (1999). Chemotactic response toward chemokines and its regulation by transforming growth factor-beta1 of murine bone marrow hematopoietic progenitor cell-derived different subset of dendritic cells. Blood 93, 3225-3232.

O'Doherty U., Peng M., Gezelter S., Swiggard W., Betjes M., Bhardwaj N. and Steinman R. (1994). Human blood contains two subsets of dendritic cells, one immunologically mature and the other immature. Immunology 82, 487-493.

Oyama T., Ran S., Ishida T., Nadaf S., Kerr L., Carbone D.P., and Gabrilovich D.I. (1998). Vascular endothelial growth factor affects dendritic cell maturation through the inhibition of nuclear factor-kappa B activation in hemopoietic progenitor cells. J. Immunol. 160, 1224-1232

Papadopoulos E., Sassetti C., Saeki H., Yamada N., Kawamura T., Fitzhugh D., Saraf M., Schall T., Blauvelt A., Rosen S. and Hwang T. (1999). Fractalkine, a CX3C chemokine, is expressed by dendritic cells and is up-regulated upon dendritic cell maturation. Eur. J. Immunol. 29, 2551-2559.

Prebeck S., Kirschning C., Durr S., da Costa C., Donath B., Brand K., Redecke V., Wagner H. and Miethke T. (2001). Predominant role of toll-like receptor 2 versus 4 in Chlamydia pneumoniae-induced activation of dendritic cells. J. Immunol. 167, 3316-3323.

Randolph G., Beaulieu S., Lebecque S., Steinman R. and Muller W. (1998). Differentiation of monocytes into dendritic cells in a model of transendothelial trafficking. Science 282, 480-483.

Reis e Sousa C., Hieny S., Scharton-Kersten T., Jankovic D., Charest H., Germain R. and Sher A. (1997). In vivo microbial stimulation induces rapid CD40 ligand-independent production of interleukin 12 by dendritic cells and their redistribution to T cell areas. J. Exp. Med. 186, 1819-1829.

Riegler S., Hebart H., Einsele H., Brossart P., Jahn G. and Sinzer C. (2000). Monocyte-derived dendritic cells are permissive to the complete replicatve cycle of human cytomegalovirus. J. Gen. Virol. 81, 393-399.

Romani L., Bistoni F. and Puccetti P. (2002). Fungi, dendritic cells and receptors: a host perspective of fungal virulence. Trends Microbiol. 10, 508-514.

Romani N., Gruner S., Brang D., Kampgen E., Lenz A., Trockenbacher B., Konwalinka G., Fritsch P., Steinman R. and Schuler G. (1994). Proliferating dendritic cell progenitors in human blood. J. Exp. Med. 180, 83-93.

Romani N., Reider D., Heuer M., Ebner S., Kampgen E., Eibl B., Niederwieser D. and Schuler G. (1996). Generation of mature dendritic cells from human blood. An improved method with special regard to clinical applicability. J. Immunol. Methods 196, 137-151.

Roncarolo M., Levings M. and Traversari C. (2001). Differentiation of T regulatory cells by immature dendritic cells. J. Exp. Med. 193, F5-9.

Sewell A. and Price D. (2001). Dendritic cells and transmission of HIV-1. Trends Immunol. 22, 173-175.

Sallusto F. and Lanzavecchia A. (1994). Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor alpha. J. Exp. Med. 179, 1109-1118.

Sallusto F., Cella M., Danieli C. and Lanzavecchia A. (1995). Dendritic cells use macropinocytosis and the mannose receptor to concentrate macromolecules in the major histocompatibility complex class II compartment: downregulation by cytokines and bacterial products. J. Exp. Med. 182, 389-400.

Schnorr J., Xanthakos S., Keikavoussi P., Kampgen E., ter Meulen V. and Schneider-Schaulies S. (1997). Induction of maturation of human blood dendritic cell precursors by measles virus is associated with immunosuppression. Proc. Natl. Acad. Sci. USA. 94, 5326-5331.

Spira A., Marx P., Patterson B., Mahoney J., Koup R., Wolinsky S., Ho D. (1996). Cellular targets of infection and route of viral dissemination after an intravaginal inoculation of simian immunodeficiency virus into rhesus macaques. J. Exp. Med. 183, 215-225

Steinbrink K., Jonuleit H., Muller G., Schuler G., Knop J. and Enk A. (1999). Interleukin-10-treated human dendritic cells induce a melanoma-antigen-specific anergy in CD8(+) T cells resulting in a failure to lyse tumor cells. Blood 93, 1634-1642.

Steinman R. and Cohn Z. (1973). Identification of a novel cell type in peripheral lymphoid organs of mice. I. Morphology, quantitation, tissue distribution. J. Exp. Med. 137, 1142-1162.

Tassaneetrithep B., Burgess T., Granelli-Piperno A., Trumpfheller C., Finke J., Sun W., Eller M., Pattanapanyasat K., Sarasombath S., Birx D., Steinman R., Schlesinger S. and Marovich M. (2003). DC-SIGN (CD209) mediates dengue virus infection of human dendritic cells. J. Exp. Med. 197, 823-829.

Urban B., Ferguson D., Pain A., Willcox N., Plebanski M., Austyn J. and Roberts D. (1999). Plasmodium falciparum-infected erythrocytes modulate the maturation of dendritic cells. Nature 400, 73-77.

Verdijk R., Mutis T., Esendam B., Kamp J., Melief C., Brand A. and Goulmy E. (1999). Polyriboinosinic polyribocytidylic acid (Poly(I:C)) induces stable maturation of functionally active human dendritic cells. J. Immunol. 163, 57-61.

Wong B., Josien R., Lee S., Sauter B., Li H., Steinman R. and Choi Y. (1997). TRANCE (tumor necrosis factor [TNF]-related activation-induced cytokine), a new TNF family member predominantly expressed in T cells, is a dendritic cell-specific survival factor. J. Exp. Med. 186, 2075-2080.

Winzler C., Rovere P., Rescigno M., Granucci F., Penna G., Adorini L., Zimmermann V., Davoust J. and Ricciardi-Castagnoli P. (1997). Maturation stages of mouse dendritic cells in growth factor-dependent long-term cultures. J. Exp. Med. 185, 317-328.

Wheatley G., McKinnon K., Iacobucci M., Mahon S., Gelber C. and Lyerly H. (1998). Dendritic cells improve the generation of Epstein-Barr virus-specific cytotoxic T lymphocytes for the treatment of posttransplantation lymphoma. Surgery 124, 171-176.

Wu S., Grouard-Vogel G., Sun W., Mascola J., Brachtel E., Putvatana R., Louder M., Filgueira L., Marovich M., Wong H., Blauvelt A., Murphy G., Robb M., Innes B., Birx D., Hayes C. and Frankel S. (2000). Human skin Langerhans cells are targets of dengue virus infection. Nat. Med. 6, 816-820.

Zhou L. and Tedder T. (1995). Human blood dendritic cells selectively express CD83, a member of the immunoglobulin superfamily. J. Immunol. 154, 3821-3835.

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