Skin Dendritic Cells in Immunity and Autoimmunity

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Dendritic cells (DC) are leukocytes that, although infrequently represented in their tissues of origin (lymphoid and solid organs and epithelia), are critically important in immunophysiology (Banchereau and Steinman, 1998; Banchereau *et al*, 2000). The central importance of DC can be attributed to their unique ability to function as antigen- presenting cells (APC) that can initiate responses in naïve T cells, and to dictate the net outcome of this interaction as it relates to the character of the immune responsess that ensue. DC in normal skin are comprised of epidermal Langerhans cells (LC) and dermal DC (DDC). In selected inflammatory conditions, several more recently described DC subpopulations can be detected and may be relevant: plasmacytoid DC (PDC) and inflammatory dendritic epidermal cells (IDEC) (Wollenberg *et al*, 2002).

The various DC subpopulations in skin can be differentiated from each other (and from non-DC) using monoclonal antibodies that react with a variety of cell surface and intracellular molecules, including differentiation antigens, major histocomopatibility (MHC) antigens, and costimulatory molecules (Shortman and Liu, 2002). It is important to distinguish the different types of skin DC because it is very likely that at least some subpopulations of skin DC have distinct functional properties. By inference, different skin DC may be differentially involved in the pathogenesis of individual skin diseases.

LC are distinguished from other DC by expression of the non-MHC class I molecule CD1a, the intercellular adhesion molecule E-cadherin, and the novel C-type lectin Langerin (Jakob and Udey, 1999; Valladeau *et al*, 2000). Langerin localizes to Birbeck granules, distinctive intracytoplasmic vesicles that have long been pathognomonic for these cells, and is expressed on cell surfaces as well. Like other DC, LC express the leukocyte (β2) integrin CD11c and MHC class II antigens, and have the capacity to upregulate a variety of costimulatory molecules (CD40, CD80, CD86, CD58, and others). In normal skin, DDC can be differentiated from LC by their location, lack of CD1a, E-cadherin and Langerin expression, absence of Birbeck granules, and expression of CD1b and CD1c.

In inflamed skin, several additional DC subpopulations have been identified (Wollenberg *et al,* 2002). IDEC are CD1a+, HLA-DR + cells that can be differentiated from LC by coexpression of high levels of the leukocyte integrin CD11b. PDC are distinguished from the other skin DC by a lack of CD11c expression and by expression of CD123 as well as the recently described surface marker BCDA-2. Like other skin DC, PDC express high levels of MHC class II antigens and can be induced to express costimulatory molecules.

ONTOGENY OF SKIN DENDRITIC CELLS

Information regarding skin DC ontogeny comes from *in vitro* experiments in which DC with characteristics of the various subpopulations are propagated in culture, *in vivo* experiments involving adoptive transfer of putative precursor cells in mice, and corroborative data from patients who receive hematologic reconstitution during cancer therapy (Shortman and Liu, 2002). Like all leukocytes, skin DC are ultimately derived from bone marrow precursors. On the basis of cell surface markers, it has been determined that LC, DDC, and IEDC are most closely related to cells of the myeloid lineage. Cells with characteristics of LC and DDC have been derived from lineage-negative CD34+ precursors *in vitro*: the former from cutaneous leukocyte antigen (CLA, PSGL-1: an E-selectin ligand)-expressing cells and the latter from CLA− cells. In serum-free medium, propagation of LC-like cells is absolutely dependent on the presence of low concentrations of transforming growth factor-β (TGF-β) (Strobl *et al*, 1996). The significance of this finding is emphasized by studies of TGF-β knockout mice that indicate that these mutant animals are devoid of LC (Borkowski *et al*, 1996).

Identification of physiologic immediate precursors of LC (as well as other skin DC) has been difficult. Because there is considerable plasticity in the DC lineage, it is possible that distinctive skin DC subpopulations may have multiple immediate precursors, or that cells may be derived from different precursor pools under steady state (resting) conditions and in the setting of skin inflammation. *In vitro* data indicate that human peripheral blood monocytes can acquire certain LC characteristics after treatment with GM-CSF and TGF-β (Geissmann *et al*, 1998) or GM-CSF and IL-15 (Mohamadzadeh *et al*, 2001). In addition, monocyte-like CD14+, Langerin + cells can be recovered from normal human skin and induced to differentiate into LC-like (CD1a+, E-cadherin +, Langerin +) cells by culturing them with TGF-β *in vitro* (Larregina *et al*, 2001). Very recent and compelling studies in mice indicate that in the absence of skin inflammation, epidermal LC are derived from radioresistant, long-lived, proliferating,

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Abbreviations: APC, antigen-presenting cell; DC, dendritic cell; IEDC, inflammatory epidermal dendritic cell; LC, Langerhans cell; MHC, major histocompatibility; PDC, plasmacytoid dendritic cell; TLR Toll-like receptor.

local precursors (Merad *et al*, 2002). This characteristic distinguishes them from all other myeloid DC. In addition, it has been determined that rapid recruitment of LC bone marrow–derived precursors occurs during skin inflammation. Whether or not these latter cells bear any relationship to the IDEC described in human skin remains to be determined.

PDC are distinct from the other skin DC both in surface phenotype (see above) and in *in vitro* growth requirements (Kadowaki and Liu, 2002). Whereas propagation of myeloid DC is dependent on, or is enhanced by, inclusion of GM-CSF in the media, PDC survival and differentiation *in vitro* is dependent on IL-3. It has been suggested that PDC may be more closely related to lymphocytes than to myeloid cells.

FUNCTION OF SKIN DENDRITIC CELLS

The role that DC play as intercellular bridges between innate and adaptive immune responses is well established (Banchereau and Steinman, 1998; Banchereau *et al*, 2000; Janeway and Medzhitov, 2002). Immature members of the lineage (such as LC) are stationed at interfaces between the organism and the outside world and are equipped with sensors that allow them to survey their microenvironment and to respond to perturbations (Pulendran *et al*, 2001). These sensors include a variety of cell surface proteins that are involved in the recognition of pathogens and pathogen-associated molecules or cell fragments (e.g., lectins, complement and Fc receptors, CD36, and αv-containing integrins) as well as molecules that are required for cellular responses to these insults (e.g., Toll-like receptors (TLR)) (Shizuo *et al*, 2001). After recognition and ingestion of complex antigens, and coincident with activation, DC degrade antigens into MHC class I and II binding peptides, upregulate costimulatory molecule expression and cytokine production, and translocate from epidermis to regional lymph nodes where they interact with naïve T cells (Banchereau *et al*, 2000). The ability of different types of DC to recognize and respond to different external stimuli is determined by the cell surface receptors that they express. Similarly, the outcome of the interaction between DC and T cells is determined by levels of MHC antigen-peptide complexes and costimulator molecules that are expressed on DC surfaces and the levels and types of cytokines that are produced. The selective expression of the nonclassical MHC class I antigens CD1a,b,c in addition to classical MHC class I and II antigens is responsible for the ability of LC, DDC, and IDEC to be efficient stimulators of T cell responses to mycobacteria-derived glycolipids (Porcelli and Modlin, 1999).

Exogenously acquired antigens is readily processed and presented to CD4 T cells in the context of MHC class II antigens. Interestingly, exogenously acquired particulate antigen (including that acquired from apoptotic cells) can be efficiently acquired and processed by DC for presentation to CD8 T cells via a process termed "cross-presentation" (Heath and Carbone, 2001). There is considerable interest in defining the cell surface receptors that play critical roles in apoptotic cell recognition and uptake and in delineating the downstream events that ensue. There is also considerable interest in identifying physiologic ligands for the myriad of C-type lectins that are expressed by dendritic cells (Figdor *et al*, 2002). One such lectin, mannan-binding DC-SIGN, has been determined to interact with ICAM-3 (and ICAM-2), HIV-associated gp120, and mycobacteria. By analogy, identification of ligands for Langerin (Valladeau *et al*, 2000) may provide insights into specialized functions of the skin DC subtype (LC) that selectively expresses this protein.

Expression of different TLRs by different skin DC will determine whether of not they participate in responses to particular noxious insults (Hornung *et al*, 2002; Kadowaki and Liu 2002). Immature human myeloid DC contain mRNAs encoding TLRs 1, 2, 3, 4, 5, 6, 8, and 10, whereas PDC synthesize TLRs 1, 6, 7, 9, and 10. Thus, engagement of TLR4 by LPS will activate myeloid DC, and the IL-12 that is produced will promote TH1 (as compared with TH2) development. Because PDC do not express TLR4, they will not respond to LPS but can release IL-12 after activation via the TLR9 ligand, bacteria-derived immunostimulatory (unmethylated CpG island–containing) DNA. PDC are also distinguished from the other skin DC by producing prodigious amounts of type I interferons in response to viral infection (Kadowaki and Liu, 2002). The unique ability of PDC to produce large amounts of type I interferons after viral challenge indicates that are they are essential participants in antiviral immunity.

It was recently proposed that DC (including skin DC) may be involved in maintenance of peripheral tolerance (Steinman and Nussenzweig, 2002). Deletion of autoreactive T cells in the thymus during embryologic development is incomplete, so central tolerance does not insure protection from autoimmune disease. The "rediscovery" of suppressor T cells, now termed "regulatory" T cells, has provided a plausible mechanism for maintenance of peripheral tolerance (McHugh and Shevach, 2002; Shevach, 2002). In addition, definition of the activation requirements of regulatory T cells implicates immature DC as important participants in this immunoregulatory mechanism. Although immature DC are not able to stimulate naïve T cells to develop into traditional CD4 or CD8 effectors, they are efficient stimulators of CD4+ CD25+ regulatory T cells that can in turn actively suppress development of effector cells (Mahnke *et al*, 2002). In this way a basal level of trafficking of LC from skin to regional lymph node, in the absence of an activating stimulus that would result in increased expression of costimulatory molecules (Geissmann *et al*, 2002). This process would allow for chronic presentation of skin-derived self antigens, such as those associated with apoptotic cells that continually generated, to autoreactive regulatory T cells, and allows for maintenance of antigen-specific unresponsiveness.

SKIN DENDRITIC CELLS AND AUTOIMMUNITY

The role (or roles) of DC in development or propagation of autoimmune diseases is (are) incompletely defined. The ability of activated DC to break tolerance to self antigens in the setting of DC cancer vaccine trials in mice and in humans (Steinman and Pope, 2002) and the emerging role for DC in the maintenance of peripheral tolerance (Steinman and Nussenzweig, 2002) suggest that DC are relevant, however. Further characterization of the activation requirements of regulatory T cells and identification of external stimuli that subvert this process will provide additional insights and may well provide additional support for the long-held concept that infectious diseases are important triggers of autoimmune diseases.

For several reasons, it is tempting to implicate skin DC as particularly relevant to autoimmune disease pathogenesis. The frequency with which skin is involved in autoimmune disease indicates that skin antigens are important targets and suggests that effector autoimmune responses may well have been initiated in skin-draining, regional lymph nodes. The recognition that blebs of apoptotic cells are rich sources of autoantigens (Casciola-Rosen *et al*, 1994) and that incident ultraviolet radiation causes keratinocyte apoptosis (Casciola-Rosen and Rosen, 1997) provides yet another possible link between autoimmunity and skin. Although the uptake of apoptotic cell-derived antigens by macrophages typically results in an anti-inflammatory response characterized by IL-10 and/or TGF-β production, in the absence of efficient clearance by macrophages, apopotic cells can clearly be immunogenic (Rosen and Casciola-Rosen, 2001). In this instance, DC are almost certainly the relevant APC and it is logical to assume that LC or DDC (or both) may be able to fulfill this role. It has also been suggested that PDC are relevant to autoimmune disease pathogenesis, particularly systemic lupus erythematosus (SLE) pathogenesis. PDC are frequently found in LE skin lesions (Farkas *et al*, 2001), and type I interferons, produced largely by PDC, have been implicated as key players in this disease (Palucka *et al*, 2002).

Increasingly, DC experts are turning their attention to elucidation of the roles that DC play in disease pathogenesis and to designing therapies that will translate increased understanding of DC physiology into improved patient care. To date, efforts to develop active immunotherapies for cancer and chronic infections have been at the forefront (Steinman and Pope, 2002). As DC-dependent mechanisms that are responsible for the avoidance of unwanted autoreactivity become better defined and as the mechanisms by which autoimmune diseases are triggered become better understood, we can anticipate that novel DC-based treatments for autoimmune diseases will be forthcoming.

Note: This brief treatise is not intended to be comprehensive. The author apologizes in advance for omissions, both planned and unintended. For in-depth treatment of the subject matter, the interested reader should refer to one or more of the excellent reviews that regularly appear (some of which are referenced herein) or the primary literature.

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