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DC-SIGN: A Guide to Some Mysteries of Dendritic Cells

Minireview

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During the last decade, dendritic cells (DCs) have come to be appreciated as critical controllers of the immune response, especially T cell responses. Although T lymphocytes actually mediate resistance to infections, transplants, and even tumors, without proper instruction from DCs, T cells would be severely compromised. DCs convert antigens from foreign cells and infectious microorganisms into short peptides that are bound to membrane proteins of the major histocompatibility complex (MHC). These MHC–peptide complexes are formed intracellularly but are ultimately presented on the plasma membrane where they serve as ligands for antigen-specific T cell receptors (TCR). In addition to TCR ligand formation, DCs carry out many other functions, some to be considered below, which allow them to control immunity at several points.

Despite their importance, the DC can be regarded as the Cinderella of the immune system, for years kept by the hearths of a few laboratories. With added attention, as illustrated by two papers by Geijtenbeek and colleagues in this issue of *Cell*, one can begin to appreciate some of the DC's glamor. Both papers center on DC-SIGN, a new DC-restricted molecule. DC-SIGN in turn qualifies as Cinderella's glass slipper, as it seems to be used by the dashing T cell and the wicked HIV-1 to identify their DC. Yet the slipper, like its wearer, had to be rescued from years of oblivion. None of the new ideas described in the papers by Geijtenbeek et al. were apparent from the genomic sequence of DC-SIGN, first reported in 1992 and since then deposited several times in gene banks. Instead the research had to shift to DCs for the importance of this molecule to be appreciated.

DC SIGN is a type II membrane protein with an external mannose-binding, C-type lectin domain. It was cloned from a placental library, through its capacity to bind the glycan-rich HIV-1 envelope in the absence of CD4 (Curtis et al., 1992), the classic virus receptor. In the first of these two papers (Geijtenbeek et al., 2000a), the lectin is rediscovered and renamed DC-SIGN, because it is a "DC-specific, *I*CAM-3 *g*rabbing, *n*onintegrin." It is proposed that the interaction of DC-SIGN with ICAM-3 establishes the initial contact of the DC with a resting T cell, helping to explain the potency with which DCs initiate T cell immunity. Potency has long been apparent in tissue culture and in experimental animals, and this adjuvant role of DCs has been extended to humans (Dhodapkar and Bhardwaj, 2000). The second paper (Geijtenbeek et al., 2000b) addresses the known capacity of DCs to promote HIV-1 infection in culture. DC-SIGN proves to be a special kind of viral receptor, promoting binding and transmission of HIV-1 to T cells, rather than viral entry into the DC. Therefore, DC-SIGN likely will be pivotal for explaining some important functions of DCs.

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The Potency of DC in Initiating Immune Responses from Resting T Cells

The term "potency" indicates that relatively small numbers of DCs, and relatively low doses of an antigen or other T cell stimulus, are sufficient to initiate rapid and strong responses, such as T cell proliferation and lymphokine production. Potency is not simply a matter of more efficient MHC–peptide complex formation, although this too is a newly recognized mechanism used by DCs to control immunity (Inaba et al., 2000). Instead potency is readily observed with stimuli that do *not* require processing, e.g., polyclonal mitogens, microbial superantigens, and transplantation antigens. DCs are also effective when the amount of membrane-bound TCR ligand is vanishingly small, as few as 100–1000 ligands on the entire cell surface (Bhardwaj et al., 1993). For these reasons, the efficacy of DCs has been attributed to special accessory molecules. Many such molecules are found on DCs, e.g., CD48, -54, -58, -80, -86, and the corresponding antibodies can block DC–T cell interactions. However, these membrane proteins are shared with other antigen-presenting cells. DC-SIGN is the first recognized DC-restricted product that helps stimulate resting T cells.

The Figdor lab realized that resting T cells expressed the adhesion molecule ICAM-3. In contrast to what was expected, ICAM-3 did not bind to β_2 integrins on DCs. When they made antibodies to block ICAM-3 binding to DCs, the monoclonals identified a small 44 kDa molecule. Its binding to ICAM-3 was Ca^{2+} dependent and blocked by mannan. The antibodies reacted specifically with DCs. Cloning showed that the ICAM-3-binding molecule was identical to the previously defined HIV-1 envelope–binding lectin (Curtis et al., 1992). In functional tests, the renamed DC-SIGN contributed to transient DC–T cell clustering and responses to transplantation antigens.

Geijtenbeek et al. (2000a) propose that DC-SIGN mediates the known loose adhesion that takes place between DCs and T cells in the apparent absence of foreign antigen (Figure 1). Such adhesion seems necessary, because MHC–peptide ligands are membrane bound, typically scarce (10–1000 copies/cell), and need to be recognized by the TCR, another membrane molecule. DC-SIGN-mediated adhesion provides an opportunity for the TCR to scan the DC surface to identify these small amounts of TCR ligand, which then activate the resting T cell. Subsequently T cells respond vigorously to antigens presented by other cells, but by then, the activated T cells are replete with their own functional adhesion molecules.

Further experiments will decipher this proposed mechanism of action and dissect DC-SIGN function in vivo. Mice deleted of the gene for DC-SIGN will be valuable, assuming there are no additional homologs. Nevertheless, it is impressive at this early stage of research to recall that human cells have been used to uncover most of the important accessory membrane proteins

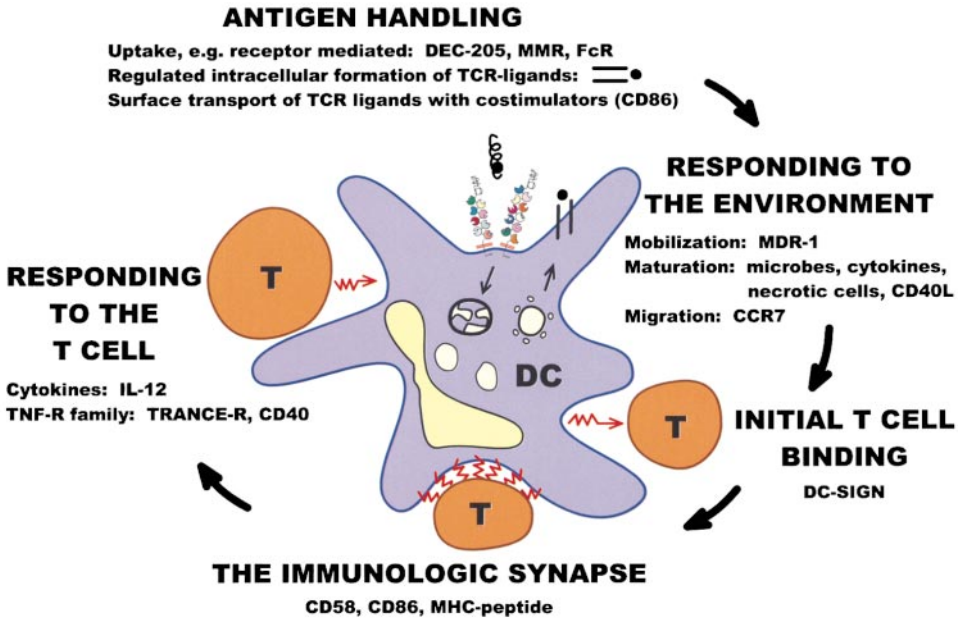

Figure 1. A Proposed Sequence of Action for the Initiation of Immunity by DCs

that control T cell function, not just DC-SIGN and ICAM-3. These include CD58 (LFA-3) for CD2; CD54 (ICAM-1) for CD11a (LFA-1); CD40L (gp39) for CD40; CD80 (B7-1) and CD86 (B7-2) for CD28 and CD154 (CTLA-4) (Figure 1).

Geijtenbeek et al. (2000a) detected DC-SIGN on DCs that were not mature or terminally differentiated, and expression of DC-SIGN did not increase when the DCs matured. This raises the possibility that DC-SIGN is mainly needed by developing DCs, not yet expressing optimal levels of MHC–peptide and accessory molecules like CD86, to contact ICAM-3 on resting T cells and then mature with help from the responding T cells. Conceivably, DC-SIGN could be involved in T cell responses other than classical immunity, such as the induction of tolerance and immune regulation.

Although DC-SIGN could be a long-sought, DC-unique molecule for T cell adhesion, and thereby help to explain the potency of DCs, there has been progress on another hypothesis. DCs are held to have other unique products to enhance signaling together with the TCR, i.e., costimulation. While DCs are remarkable in this regard, to date costimulation is not known to involve a DC-specific product. Instead, the potent costimulator, CD86, is abundant on DCs relative to other antigen-presenting cells. More remarkably, during the vesicular transport of newly formed MHC–peptide complexes in developing DCs, the complexes move together with CD86. Upon arrival at the DC surface, MHC–peptide and CD86 are deposited as stable clusters (Inaba et al., 2000). Therefore DCs are designed to set up the "immunological synapse."

The concept of a synapse, a term first coined by William Paul, proposes a central contact zone in which the APC and T cell membranes are only 134 Å apart (Davis and van der Merwe, 1996; Shaw and Dustin, 1997). The zone contains multiple copies of molecular couples that span this distance, e.g., CD48 or -58 plus CD2, CD80 or -86 plus CD28, and importantly, MHC–peptide plus TCR (Figure 1). This assembly of supramolecular aggregates may facilitate costimulation and sustain the low affinity interaction between TCR ligands and the TCR. Direct experiments on synapses have so far used activated T cells. DCs might allow the concept to be pursued in naive T cells. In sum, more precise mechanisms are beginning to explain DC function: DC-SIGN for T cell adhesion, and preformed aggregates of MHC–peptide and membrane accessories for costimulation.

We have only been discussing the basis for DC potency in vitro. In vivo, DCs are found in peripheral tissues, such as the skin and airways but they can migrate to lymphoid tissues. There, in the T cell areas, the DCs are in full view of the circulating, naive lymphocyte repertoire and can even make chemokines that attract these T cells (Adema et al., 1997). The match of the DC and T cell can then be made via DC-SIGN, allowing the intimate cross-talk between the two cells to begin (Ingulli et al., 1997). Nonetheless, to control immunity, the DC displays other functions prior to the use of DC-SIGN. In the peripheral outposts for antigen entry, DCs are immature requiring a stimulus, such as exposure to microbial products or inflammatory cytokines, for terminal differentiation (Cella et al., 1997). The immature DCs express several receptors for antigen uptake, including other lectins (Figure 1, top). The endocytic system is in turn regulated by a maturation stimulus to efficiently convert antigens to MHC–peptide complexes, in concert with CD86 costimulators as mentioned above (Inaba et al., 2000). DC migration from the periphery to the lymphoid tissues involves mobilization via multidrug resistance receptors (Randolph et al., 1998) and chemotaxis through the CCR7 receptor toward chemokines produced in lymphoid tissues and lymphatic channels (Forster et al., 1999). After DC-SIGN functions, the synapse forms and the resting T cell is activated. Then TNF family members

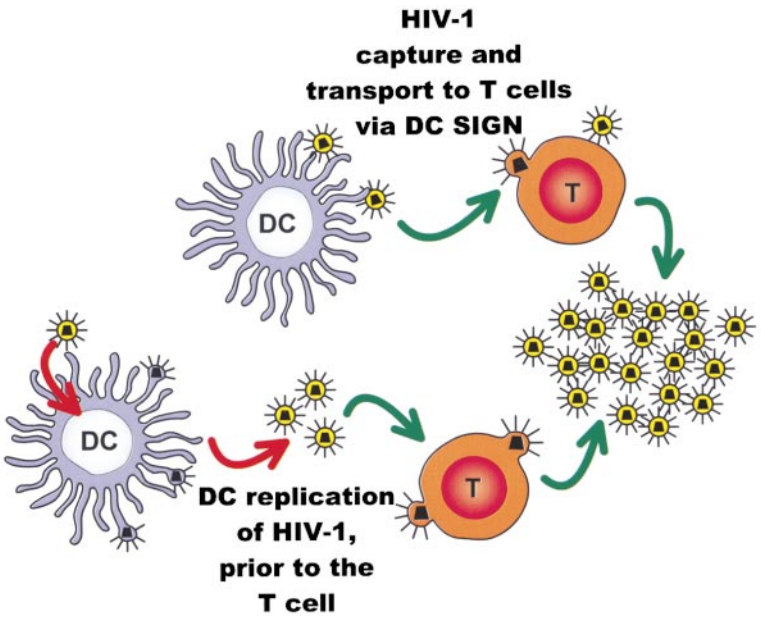

Figure 2. Proposed Pathways for the Transmission of HIV-1 or SIV to Permissive T Cells

The upper, DC-SIGN-dependent pathway does not distinguish M-tropic from T-tropic HIV-1. It may operate, for example, on DCs beneath mucosal epithelial surfaces and is the major pathway in the cells studied by Geijtenbeek et al. (2000b). The lower, infection-dependent pathway may pertain to DCs within mucosal epithelia. Both pathways also could operate in acute and chronic phases of infection in lymphoid tissues.

on the T cell, like CD40L and TRANCE, prolong DC survival and cytokine production (Josien et al., 2000), especially the IL-12 needed for strong T cell–mediated immunity (Figure 1). Therefore DC potency is not due to one surface or secreted molecule. It results from many well-timed and spatially organized specializations.

DCs and the Transmission of HIV-1

The second of the two papers (Geijtenbeek et al., 2000b) reveals a new way for HIV-1 to exploit the DC. The investigators describe a fascinating DC-SIGN-dependent mechanism. This lectin can capture HIV-1 at low external titres. Without allowing viral entry, DC-SIGN retains the attached virus in an infectious state for days and then transmits it to replication-permissive T cells. The in vitro data are fortified with micrographs of tissue sections. DC-SIGN is found on dendritic profiles beneath genital epithelium, a major potential site for HIV-1 transmission, and in the T cell areas, the sites for viral replication especially in acute infection. This pure delivery role for DC-SIGN is consistent with data that, in lymphoid tissues, HIV-1 and SIV mainly replicate in $CD4^+$ T cells, not DCs (Stahl-Hennig et al., 1999).

The new experiments use a standard system to study the involvement of DCs in HIV-1 transmission (Pope et al., 1994). The model is to add HIV-1 to cultured DCs for 1–2 hr, wash, and at varying times, add in T cells and follow the levels and cellular sites of viral replication. A vigorous infection occurs, primarily in T cells. In such a model, antibodies to DC-SIGN exerted a significant but sometimes incomplete block of transmission. When transfected cells were used to pursue the relative roles of DC-SIGN and more classical HIV-1 receptors, DC-SIGN was not an entry receptor and did not influence the entry role of CD4 and CCR5. However, DC-SIGN on one transfectant captured virus, even when present in small amounts, and transmitted the HIV-1 to CD4 and CCR5 on other cells (Figure 2, top). DC-SIGN literally "presents" HIV-1 to T cells, but in a nonprocessed infectious form.

It is possible that other pathogens are also transmitted via DCs and in particular via DC-SIGN. The glycan ligands for this lectin could be present on other viral envelopes, the cell walls of other microbes, or even tumor cells. Also, because DC-SIGN retains its HIV-1 ligand in a native state, this and other lectins could present vaccines to protective B cells, which must react to native antigens. If the vaccine were simultaneously processed and presented to helper T cells, DC-SIGN would even set up an effective DC–T–B "ménage à trois," capable of inducing strong immunity, including mucosal immunity (Fayette et al., 1997).

DC-SIGN is not the only attraction that HIV-1 finds in DCs. The virus can infect certain DCs in culture (Figure 2, bottom, red arrows). HIV-1 is capable of replication in immature DCs, and possibly mature DCs that are interacting with CD40L or T cells (Granelli-Piperno et al., 1999). So while DC-SIGN can transport HIV-1 and enhance infection of T cells, locally or in lymphoid tissues, direct infection could also amplify the amount of virus that DCs deliver. Both pathways (Figure 2) may enhance the overall pathogenesis of HIV-1 infection, although Geijtenbeek et al. (2000b) observe that DC-SIGN in their cells plays the major role in viral replication, especially at low doses of HIV-1.

Importantly, it is well established that M-tropic strains of HIV-1 are preferentially transmitted among humans. One possible explanation is that selection takes place at the level of DC infection. By contrast, DC-SIGN ferries both M- and T-tropic viruses to T cells. Geijtenbeek et al. (2000b) did not detect DC-SIGN on DCs (Langerhans cells) within the genital epithelium, only on DCs beneath the surface. These two subsets of DCs, termed "epidermal or epithelial" and "dermal or subepithelial," represent distinct pathways of differentiation (Caux et al., 1996). Since epidermal, DC-SIGN-negative DCs likely select for M-tropic HIV-1 (Reece et al., 1998), these DCs in vivo may account for the selective transmission of M-tropic HIV-1, which then binds to additional subepithelial, DC-SIGN-positive DCs, greatly amplifying delivery

of virus to T cells locally and eventually lymphoid tissue. Beyond these functions in HIV-1 capture and conveyance, DCs can serve another nefarious role, activating T cells to be permissive for HIV-1 replication (Figure 2, right).

Implications

A new DC-restricted molecule, DC-SIGN, demystifies two of this cell's contrasting functions: stimulating T lymphocytes to develop immunity, and enhancing HIV-1 and SIV replication. For immunity, it is implied that DC-SIGN allows the DC to interact temporarily with naive T cells. Critical events of antigen recognition can then ensue, leading to the formation and function of a contact zone termed the immunological synapse, rich in interacting adhesion and signaling molecules. For immunodeficiency viruses, DC-SIGN enables the DC to bind and transmit virus to permissive T cells. This should occur in vivo beneath the genital epithelium and in the T cell areas of lymphoid tissues, because of DC-SIGN expression in these sites. DC-SIGN may allow DCs to carry additional pathogens to their cellular targets. The new data identify DC-SIGN as a potential site on DCs for manipulating both the immune response and HIV-1 infection. The results illustrate a larger issue. To understand and manipulate immune responsiveness, and many clinical areas involving the immune system, one should not restrict the analysis to antigens and lymphocytes. One must also consider DCs, the captivating controllers of immunity.

Acknowledgments

The author regrets that, because of space limitations, many valuable references could not be included.

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