The Journal of Immunology

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J Immunol 2002; 168:2118-2126; ;
doi: 10.4049/jimmunol.168.5.2118
http://www.jimmunol.org/content/168/5/2118

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852

Print ISSN: 0022-1767 Online ISSN: 1550-6606.

The Dendritic Cell-Specific Adhesion Receptor DC-SIGN Internalizes Antigen for Presentation to T Cells[1]

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Dendritic cells (DCs) capture Ags or viruses in peripheral tissue to transport them to lymphoid organs to induce cellular T cell responses. Recently, a DC-specific C-type lectin was identified, DC-specific ICAM-grabbing non-integrin (DC-SIGN), that functions as cell adhesion receptor mediating both DC migration and T cell activation. DC-SIGN also functions as an HIV-1R that captures HIVgp120 and facilitates DC-induced HIV transmission of T cells. Internalization motifs in the cytoplasmic tail of DC-SIGN hint to a function of DC-SIGN as endocytic receptor. In this study we demonstrate that on DCs DC-SIGN is rapidly internalized upon binding of soluble ligand. Mutating a putative internalization motif in the cytoplasmic tail reduces ligand-induced internalization. Detailed analysis using ratio fluorescence imaging and electron microscopy showed that DC-SIGN-ligand complexes are targeted to late endosomes/lysosomes. Moreover, ligands internalized by DC-SIGN are efficiently processed and presented to $CD4^+$ T cells. The distinct pattern of expression of C-type lectins on DCs in situ and their nonoverlapping Ag recognition profile hint to selective functions of these receptors to allow a DC to recognize a wide variety of Ags and to process these to induce T cell activation. These data point to a novel function of the adhesion receptor DC-SIGN as an efficient DC-specific Ag receptor that can be used as a target to induce viral and antitumor immunity. *The Journal of Immunology,* **2002, 168: 2118–2126.**

Dendritic cells (DCs)[3] have the unique capacity to initiate immune responses in lymphoid organs against Ags derived from peripheral sites (1). In tissues, like skin and mucosa, immature DCs sample a broad variety of Ags via fluid phase uptake and receptor-mediated endocytosis (2, 3). Internalized Ags are processed along the endosomal/lysosomal pathway for subsequent formation of MHC class II-peptide complexes (4). Upon inflammation-induced migration of DCs to secondary lymphoid organs, Ag sampling ceases and MHC-peptide complexes are redistributed from intracellular organelles to the cell surface (5, 6). Besides high expression of MHC molecules, mature DCs express high levels of costimulatory and adhesion molecules for efficient activation of resting T lymphocytes (7).

Several receptors expressed by immature DCs belong to the C-type lectin superfamily, including Langerin (CD207), the mannose receptor (MR; CD206), and DEC-205 (CD205) (8). C-type lectins are characterized by a carbohydrate recognition domain (CRD) that interacts with proteins with either mannose or galactose side chains in a calcium-dependent manner (9). The C-type lectins on DCs have a mannose-type specificity, and binding of mannosylated ligands can be blocked by mannan. However, the number of CRDs present in these lectins differs and the complexity of the mannose groups that they recognize is distinct (9, 10). Langerin contains a single CRD and functions on skin epidermal Langerhans cells (LCs) as an endocytic receptor that is associated with and induces formation of Birbeck granules (11, 12). The MR, highly expressed on immature DCs and macrophages, has eight potential CRDs, of which two were shown to be functional for binding end-standing mannose groups on a variety of pathogens and Ags, illustrating its role in innate immunity (13, 14). The MR is constitutively internalized from the cell surface into early endosomes and recycles back to the plasma membrane (15). During this recycling process, bound ligand is released from the MR in the acidic environment of the endosomes for subsequent processing, resulting in high amounts of internalized Ags and efficient Ag presentation by DCs (16, 17). Another multilectin endocytic receptor is DEC-205, which contains 10 CRDs and is expressed both by DCs and by thymic epithelial cells (18, 19). To date, the mannosylated ligands recognized by DEC-205 are not identified. The cytoplasmic tail of DEC-205 contains sequences that regulate its targeting and recycling (20). Both the MR and DEC-205 contain a tyrosine-based motif for internalization in clathrin-coated vesicles (21, 22). Whereas the MR traffics through early endosomes, DEC-205, on the contrary, recycles deeper into the endosomal/lysosomal pathway through MHC class II compartments by means of a triacidic cluster in its cytoplasmic tail (20).

We recently identified a novel C-type lectin, DC-specific ICAM-grabbing non-integrin (DC-SIGN; CD209), that has a single CRD with mannose-type specificity and is exclusively expressed on DCs, in contrast to the MR and DEC-205, which are also expressed on other cell types (23). Interestingly, the cytoplasmic tail of DC-SIGN contains a triacidic cluster, as well as two putative internalization motifs. However, DC-SIGN has been reported to be a cell adhesion receptor that mediates cell interactions

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Received for publication November 2, 2001. Accepted for publication December 21, 2001.

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[1] This work was supported by Heart Foundation Grant 97.078 (to A.E.), AIDS Foundation Grant 5008 (to T.B.H.G.), and a Leenaards Foundation grant (to V.P.).

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[3] Abbreviations used in this paper: DC, dendritic cell; MR, mannose receptor; CRD, carbohydrate recognition domain; LC, Langerhans cell; DC-SIGN, DC-specific ICAM-grabbing non-integrin; PFA, paraformaldehyde.

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between DCs and resting T cells upon binding its counterstructure ICAM-3 (23). DC-SIGN also displays a high affinity for ICAM-2, supporting transendothelial migration of DCs and DC trafficking (24). Besides the cellular ligands ICAM-2 and ICAM-3, DC-SIGN binds the HIV-1 envelope glycoprotein gp120 (25, 26). Interestingly, DC-SIGN captures HIV-1 at mucosal sites of initial infection and protects the virus from degradation for subsequent transport by DCs to lymphoid organs. Here, DC-SIGN efficiently transmits the virus to T lymphocytes, resulting in productive HIV infection of T cells (26). The mechanism of protection of the virus by DCs is currently unknown, but several studies have demonstrated intracellular localization of virions in DCs (27). This, together with the fact that the cytoplasmic tail of DC-SIGN contains internalization motifs, prompted us to analyze the intracellular routing of DC-SIGN upon binding of soluble ligand. We demonstrate that DC-SIGN is indeed internalized from the cell surface upon ligand binding. Detailed analysis revealed that DC-SIGN-ligand complexes are targeted to late endosomes/lysosomes. Moreover, Ag internalization by DCs via DC-SIGN resulted in efficient Ag presentation to a $CD4^+$ T cell clone.

Materials and Methods

Antibodies

The following Abs were used: AZN-D1, AZN-D2, and AZN-D3 (IgG1, anti-DC-SIGN (23, 24)); DCN46.1 (IgG2b, anti-DC-SIGN; kind gift of Dr. M. Colonna, Washington University, St. Louis, MO); CSRD (polyclonal antiserum obtained after immunization of rabbits with the following peptide from DC-SIGN coupled to keyhole limpet hemocyanin: CSRDEEQFLSPAPATPNPPPA); L19 (anti-CD18 (28)); AZN-ICM2.1 (IgG1, anti-ICAM-2 (29)); 3.29B1 (IgG1, anti-MR; kind gift of Dr. M. Cella, Washington University); DCGM4 (anti-Langerin; Beckman Coulter, Fullerton, CA); and anti-DEC-205 (kind gift of Dr. R. Steinman, Rockefeller University, New York, NY (30)).

Cells

Human blood monocytes were isolated from buffy coats by adherence or by anti-CD14 microbeads (Miltenyi Biotec, Auburn, CA). Immature DCs were generated by culturing monocytes in RPMI 1640/10% FCS in the presence of IL-4 (500 U/ml; Schering-Plough, Kenilworth, NJ) and GM-CSF (800 U/ml; Schering-Plough) for 5–8 days. For maturation, cells were cultured for an additional 40 h in the presence of 2 μg/ml LPS (from *Salmonella typhosa*; Sigma-Aldrich, St. Louis, MO). Alternatively, CD34-derived DCs were cultured as described (31, 32). Cells were matured using 20 ng/ml LPS for 24 h, and their phenotype was verified by analysis of the expression of CD83 and CD86.

Immunohistochemistry

Cryosections (4 μm) of the tissues were fixed in acetone for 10 min and incubated with primary and secondary Abs as described (23). Final staining was performed with the ABC-PO/ABC-AP Vectastain kit (Vector Laboratories, Burlingame, CA). Counterstaining was performed with hematoxolin.

Ultrastructural localization studies were performed on immature DCs incubated with AZN-D2 at 4°C. After 0 and 30 min at 37°C, cells were fixed in 1% paraformaldehyde (PFA) in 0.1 M phosphate buffer for 2 h at room temperature. Fixed cells were stored until use in 1% PFA. Before sectioning, cells were pelleted in 10% gelatin and postfixed in 1% PFA for 24 h. Ultrathin cryosectioning was performed as described before (33, 34). Sections were incubated with a rabbit anti-mouse antiserum to visualize the internalized AZN-D2 Abs followed by protein A complexed with 5 nm gold. Subsequently, sections were incubated with CSRD followed by protein A complexed to 10 nm gold. Electron microscopy was performed using a JEOL 1010 electron microscope (JEOL, Tokyo, Japan) operating at 80 kV.

Immunofluorescence

Cells were stained in PBS/0.5% BSA with primary Abs and FITC-conjugated secondary Abs and were analyzed by flow cytometry using the FACScan (BD Biosciences, Mountain View, CA). Isotype-specific controls were included.

Alternatively, cells were fixed in 1% PFA in PBS for 30 min and were permeabilized in PBS/0.5% saponin before staining. After staining, cells were allowed to adhere to poly-L-lysine-coated glass slides, mounted in antibleach reagent, and analyzed using an MRC600 confocal microscope (Bio-Rad, Hercules, CA).

Binding and uptake of mannosylated BSA

Immature DCs were preincubated for 15 min at 37°C with mannan (100 μg/ml), EDTA (2.5 mM), AZN-D1, or AZN-D2 (20 μg/ml) in Tris sodium albumin (TSA) (20 mM Tris-HCl (pH 8.0), 150 mM NaCl, 1 mM $CaCl_2$, 2 mM $MgCl_2$, and 0.5% BSA). Mannosylated biotinylated BSA (10 μg/ml; Sigma-Aldrich) was added. After 1 h at 37°C, cells were washed, fixed, and permeabilized as for immunofluorescence. Cells were stained using avidin-FITC and were analyzed using flow cytometry.

To analyze the ability of mannosylated BSA to block ICAM-3 binding to DC-SIGN, DCs were preincubated for 15 min at 37°C as indicated and ICAM-3-coated TransFluorSpheres (Molecular Probes, Eugene, OR) were added. Alternatively, for determining pH dependency of binding to gp120-coated beads, DCs were preincubated in TSA with different pH and were kept in this buffer throughout the assay. The fluorescent bead adhesion assay was performed as described previously (23).

Internalization assay

Immature DCs were incubated with Abs or soluble ICAM-Fc chimeras in TSA for 1 h on ice and were subsequently washed. Cells were incubated at 37°C for various times, placed on ice, and incubated with FITC-conjugated secondary Abs. To control for off rate of Abs at 37°C, cells were fixed before Ab binding to prevent membrane transport. Cells were analyzed using flow cytometry, and the relative difference in mean fluorescence intensity compared with fixed cells was calculated.

pH measurement

The pH of the organelles to which internalized DC-SIGN-ligand complexes were targeted was measured by ratio fluorescence imaging of a pH-sensitive secondary Ab (35). DCs were incubated with anti-DC-SIGN Abs (AZN-D1), followed by FITC-conjugated secondary Abs at 4°C. Subsequently, they were allowed to adhere on cover slips coated with poly-L-lysine (Sigma-Aldrich) for 45 min at 37°C, and dual excitation ratio imaging was performed as described (35, 36). Images were acquired for 500 ms at 490 $\pm$ 6 nm and 1 s at 440 $\pm$ 6 nm using a previously described axiovert microscope (Zeiss, Oberkochen, Germany) equipped with a charged coupled device camera (36). Ratio and quantification was obtained using the Metamorph/Metafluor software (Universal Imaging, Downingtown, PA). Data analysis and statistics were performed as described (36).

Ag presentation

Hd7, a $CD4^+$ T cell clone that recognizes a peptide derived from mouse IgG1 Abs in HLA-DR0101/DQw1, was used (37). Immature DCs (20,000 cells/well) from a typed donor were preincubated with serially diluted Abs and cocultured with 80,000 T cells. After 48 h, [^{3}H]thymidine was added (0.2 μCi/well; Amersham, Arlington Heights, IL), and incorporation was measured after 16 h.

Results

DC-SIGN and the other C-type lectins Langerin, MR, and DEC-205 show distinct in vitro and in vivo distribution

To analyze whether the C-type lectins DC-SIGN, Langerin, MR, and DEC-205, which all recognize mannose-like carbohydrates, are differentially expressed on DC subsets, their in vitro and in vivo distributions were studied. Of these C-type lectins, only DC-SIGN is specifically expressed on DCs. Immunohistochemical analysis of these C-type lectins in peripheral tissues such as skin and mucosa, which contain immature DCs that function as sentinels to capture Ags or pathogens, is demonstrated in Fig. 1. Consecutive sections were stained to analyze coexpression of C-type lectins on the same cells. In skin, dermal DCs were found to express DC-SIGN as well as the MR (Fig. 1) (23). The MR was also expressed on macrophages. Langerin expression was detected on DCs in the epidermis, the so-called LCs (Fig. 1) (11), whereas DEC-205 was not detected on dermal DCs or on LCs, but instead was found on cells located in the stratum basalis, the innermost layer of the epidermis (Fig. 1). Similarly, in the uterus mucosa,

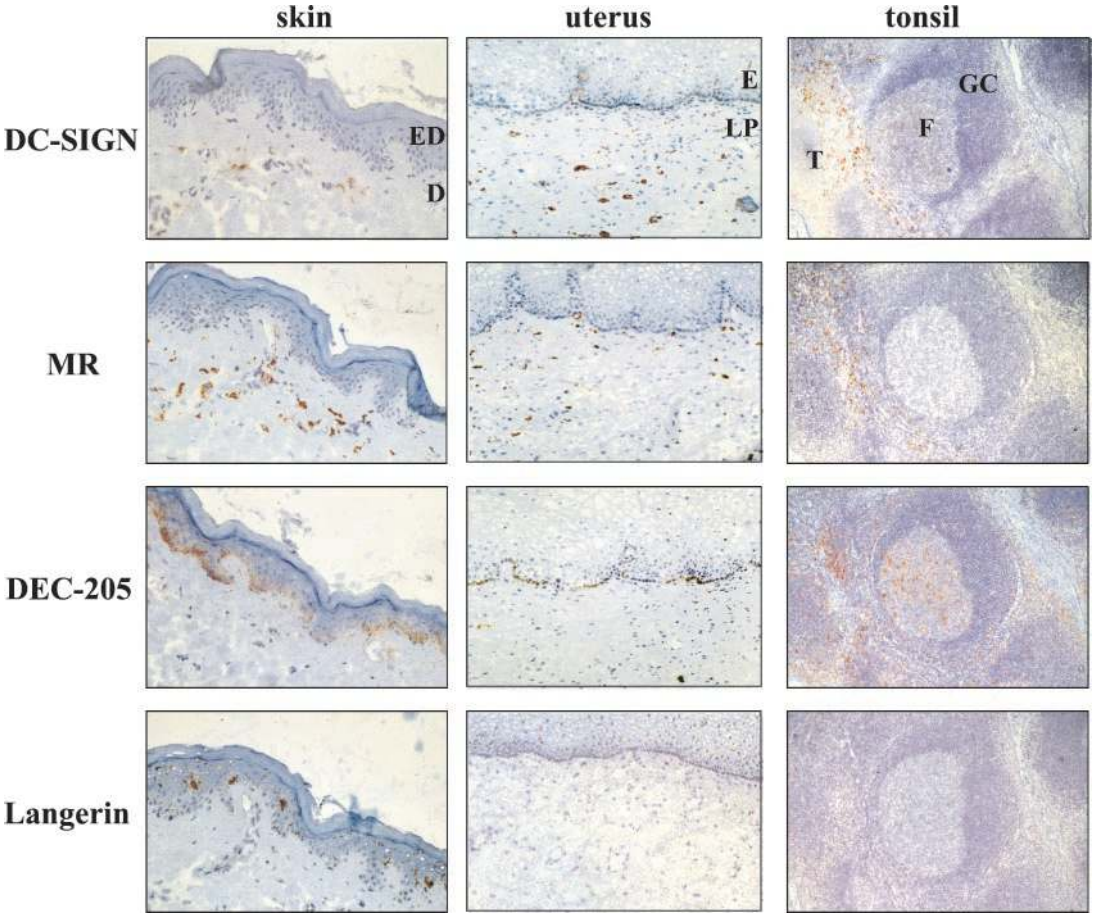

FIGURE 1. DC-SIGN is coexpressed with the MR on DCs in skin dermis, mucosal lamina propria, and tonsilar T cell areas. Distribution of DC-SIGN, the MR, DEC-205, and Langerin was analyzed on tissue sections of skin, uterus, and tonsil by immunohistochemistry. ED, Epidermis; D, dermis; E, epithelium; LP, lamina propria; T, T cell area; F, follicle; GC, germinal center.

DC-SIGN and the MR were coexpressed on lamina propria DCs (Fig. 1) (23), whereas Langerin expression could not be detected (Fig. 1). Comparable to its expression in skin, DEC-205 was detected on cells lacking DC morphology in the stratum basalis of the epithelium (Fig. 1).

To determine the expression of these C-type lectins in secondary lymphoid organs, sites of Ag presentation by mature DCs, tonsils were analyzed. Similarly as in peripheral tissues, in tonsil DC-SIGN and the MR were readily detected on DCs in T cell areas (Fig. 1) (23). Besides expression on DCs, the MR was also expressed on other cells, most likely macrophages. Expression of DEC-205 was demonstrated in T and B cell areas, including germinal centers (Fig. 1), demonstrating that DEC-205 is expressed on different DC subsets than DC-SIGN and the MR. In agreement with previous reports, Langerin expression could not be detected in tonsil (Fig. 1) (11).

In addition to their distribution in situ, the expression of C-type lectins was studied on monocyte-derived DCs generated in vitro. Immature DCs were generated by culturing human blood monocytes in the presence of GM-CSF and IL-4 and were subsequently matured in the presence of LPS for 40 h, as demonstrated by the induction of the maturation marker CD83 (Fig. 2*A*). Cell surface expression of both DC-SIGN and the MR was induced during DC differentiation and moderately down-regulated upon maturation (Fig. 2*A*) (23). In contrast, DEC-205 expression was readily detected on monocytes and increased upon maturation of DCs. Langerin expression could not be detected on monocyte-derived DCs (Fig. 2*A*) (11).

To study steady-state distribution of C-type lectins intracellularly and on the cell surface, immature and mature DCs were stained for these receptors after fixation and permeabilization and were analyzed by confocal microscopy. DC-SIGN mainly localized at the plasma membrane of DCs (Fig. 2*B*). In contrast, the MR was predominantly present in numerous intracellular vesicles (Fig. 2*B*); previous studies have shown that these organelles represent early endosomes (16, 17). Although the level of expression of DC-SIGN and the MR was reduced upon maturation of DCs, no alteration in subcellular distribution of these proteins was observed. In contrast, upon maturation DEC-205 redistributed from intracellular sites to the plasma membrane (Fig. 2*B*).

Taken together, these data demonstrate that DC-SIGN and the MR are coexpressed on both immature and mature DCs in vitro and in situ. However, these receptors differ in their subcellular distribution because DC-SIGN localizes at the cell surface, whereas the MR is present mainly in intracellular compartments, indicating different routing.

Ligand specificity of DC-SIGN differs from that of the MR

To date, three ligands of DC-SIGN have been identified, i.e., the cell surface adhesion receptors ICAM-2 and ICAM-3 and the HIV-1 envelope glycoprotein gp120. In contrast, well-known ligands for the MR are pathogens and Ags with terminal mannose groups, such as mannosylated BSA. Ligand binding to both DC-SIGN and the MR can be blocked by mannan, indicating that recognition of carbohydrates is essential for both C-type lectins. To analyze whether DC-SIGN could function similarly to the MR as a receptor for proteins containing end-standing mannoses, we incubated DCs with mannosylated BSA, consisting of BSA to which

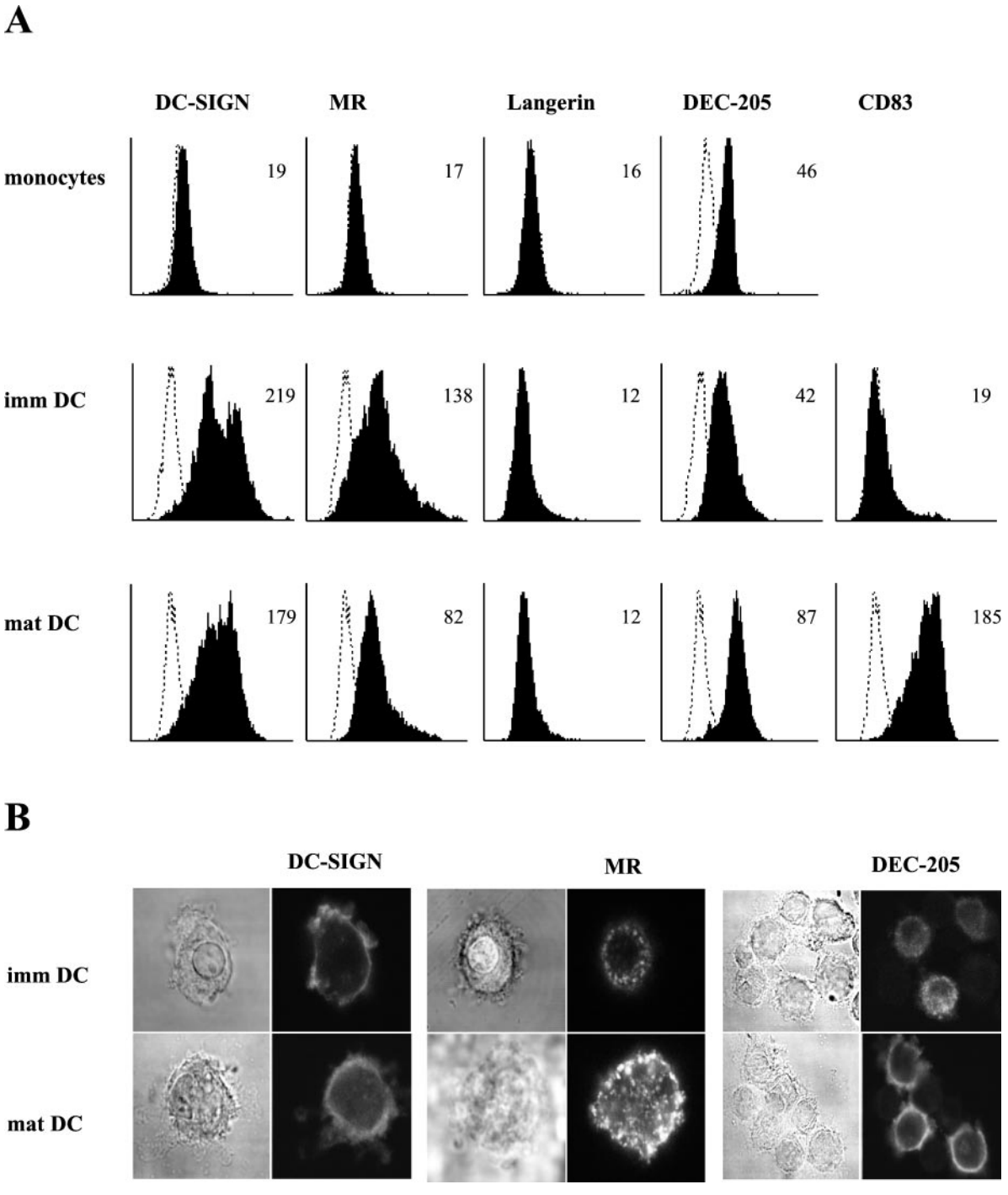

FIGURE 2. DC-SIGN localizes to the cell surface of monocyte-derived immature DCs, whereas the MR and DEC-205 are mainly distributed intracellularly. *A*, Cell surface distribution of C-type lectins. Monocytes and immature and mature DCs were stained with Abs against DC-SIGN, the MR, DEC-205, or Langerin (filled histograms) or with control Abs (open histograms) and analyzed by flow cytometry. Mean fluorescence intensity is indicated. *B*, Cell surface and intracellular staining of DC-SIGN, the MR, and DEC-205 in immature and LPS-matured DCs by confocal microscopy analysis after permeabilization of cells (*right panels*). In the *left panels*, the corresponding phase-contrast images of the cells are shown.

~15 single mannose groups are attached. Surprisingly, no mannosylated BSA could be detected on the cell surface of DCs (Fig. 3*A*). In contrast, high amounts of mannosylated BSA were readilyinternalized by DCs. This uptake of mannosylated BSA could be completely inhibited by preincubation with either mannan or EDTA (Fig. 3*A*), whereas anti-DC-SIGN Abs that recognize the CRD did not affect internalization (Fig. 3*A*; AZN-D1 and AZN-D2). This indicates that internalization of mannosylated BSA is not mediated by DC-SIGN but probably completely by the MR (16). Similarly, binding and internalization of mannosylated BSA by DC-SIGN was not observed in DC-SIGN-transfected K562 cells that lack expression of the MR (data not shown). Also, binding of DC-SIGN to ICAM-3 beads was not inhibited by preincubation with mannosylated BSA, even at high concentrations, whereas mannan and anti-DC-SIGN Abs blocked the DC-SIGN-ICAM-3 interaction (Fig. 3*B*). This is in agreement with a recent publication of Drickamer et al. (10), which shows that DC-SIGN preferentially binds high-mannose oligosaccharides. Thus, DC-SIGN and the

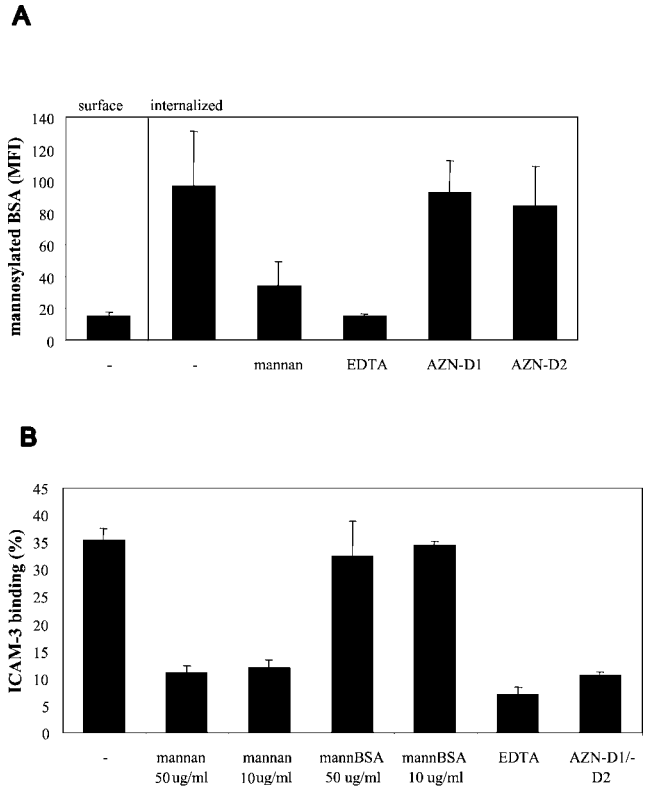

FIGURE 3. Ligand specificity of DC-SIGN differs from that of the MR. *A*, Uptake of mannosylated BSA by immature DCs after preincubation with mannan, EDTA, or anti-DC-SIGN Abs (AZN-D1 or AZN-D2). The amount of mannosylated BSA on the surface as well as intracellularly was analyzed by flow cytometry. Values represent mean of duplicates ± SD. One representative experiment of three is shown. *B*, Binding to ICAM-3 beads by immature DCs after pretreatment as in *A*. The percentage of cells that bound ICAM-3-coated fluorescent beads is depicted. Values represent mean of duplicates ± SD. One representative experiment of three is shown.

MR recognize different mannose moieties, whereas the MR has a high affinity for Ags with single mannose groups, and DC-SIGN binds more complex mannose residues.

DC-SIGN is rapidly internalized from the cell surface upon ligand binding

Several C-type lectins on DCs function as endocytic receptors and are either constitutively internalized from the cell surface, like the MR, or internalized upon ligand binding. The presence of three putative internalization and targeting motifs in the cytoplasmic tail of DC-SIGN, a tyrosine-based motif, a triacidic cluster, and a dileucine motif (Fig. 4*A*) prompted us to study whether DC-SIGN could function in endocytosis. Binding of a polyclonal antiserum (CSRD) to the C-terminal part of DC-SIGN, outside the CRD, did not lead to internalization of the receptor, even after prolonged periods of time (Fig. 4*B*). Interestingly, the mAbs AZN-D1, AZN-D2, and AZN-D3 (Fig. 4*B* and data not shown), which bind to the CRD of DC-SIGN and thus mimic ligand binding, were rapidly internalized with similar kinetics compared with the MR (Fig. 4*B*). Abs against CD18 that bind with high affinity to the adhesion receptors LFA-1, Mac-1, and p150,95, which lack any known internalization motifs, were not endocytosed by DCs (Fig. 4*C*). These Abs are of the same IgG isotype as DC-SIGN, indicating that internalization of DC-SIGN is specific and not mediated via FcR.

Next, we investigated the ability of DC-SIGN to internalize soluble ligands such as ICAM-2- and ICAM-3-Fc, which interact with DC-SIGN in a carbohydrate-dependent manner (23). DC-SIGN-bound ICAM-2-Fc and ICAM-3-Fc ligands were rapidly internalized at 37°C, with even faster kinetics compared with AZN-D2 Abs (Fig. 4*C*). Binding of soluble ligand resulted in a higher amount of DC-SIGN internalized than after triggering of endocytosis using AZN-D2 Abs. As a control, ICAM-1-Fc, which does not bind DCs through DC-SIGN, was not internalized (data not shown), illustrating again that FcR-mediated binding was not involved in the internalization of the DC-SIGN-ligand complex. After maturation, endocytosis of DC-SIGN was still taking place, although to a lower extent compared with immature DCs (Fig. 4*D*).

To analyze the contribution of the cytoplasmic tail in regulating DC-SIGN internalization, K562 cells were transfected with DC-SIGN, in which the leucines are replaced by alanines (LL/AA). Whereas wild-type DC-SIGN is efficiently internalized in K562 upon binding AZN-D2 Abs, the LL/AA mutant is endocytosed to a much lower degree (Fig. 4*E*). Thus, whereas DC-SIGN is not constitutively endocytosed, ligand binding induces internalization of DC-SIGN, which requires the dileucine motif in the cytoplasmic tail of DC-SIGN.

Internalized DC-SIGN-ligand complexes are targeted to late endosomes/lysosomes

To study the fate of internalized DC-SIGN-ligand complexes, the pH of the organelles with DC-SIGN-ligand complexes was quantified by ratio fluorescence imaging of internalized pH-sensitive FITC-conjugated anti-DC-SIGN Abs (35). Internalization of DC-SIGN-Ab complexes at 37°C demonstrated that in immature DCs these complexes localize to different sites compared with mature DCs, which have down-regulated their Ag-processing machinery. Clearly, in immature DCs the DC-SIGN-ligand complexes internalize into large intracellular compartments with low pH, in the vicinity of the nucleus (Fig. 5*A*, depicted in blue). In contrast, in mature DCs these complexes are not routed to these deeper-located compartments but reside in small organelles with a neutral pH located in proximity of the cell surface (Fig. 5*A*, depicted in green/yellow). Quantification of the pH of 804 organelles in immature DCs and 750 in mature DCs revealed that, in immature DCs, the mean pH value of the DC-SIGN-ligand-containing subcellular compartment was 5.47 (Fig. 5*B*), indicating that these are lysosomes in which degradation of ligand can occur. In mature DCs, these compartments had a mean pH of 6.45, which is indicative of early endosomes (Fig. 5*B*).

To determine whether the DC-SIGN-Ab complexes dissociate upon internalization, we followed both DC-SIGN and the Abs upon internalization into immature DCs by electron microscopy. Internalization was triggered with AZN-D2, and at different time points cells were fixed and AZN-D2 was detected with 5 nm gold. Moreover, DC-SIGN molecules were detected after fixation with polyclonal Abs (CSRD) labeled with 10 nm gold (Fig. 5*C*). At steady state, DC-SIGN localizes at the plasma membrane and cytoplasmic extensions (Fig. 5*CA*, arrowheads). No staining was observed in intracellular organelles (Fig. 5*CA*, arrows). As expected, AZN-D2 Abs were detected only at the plasma membrane directly after binding at 4°C (Fig. 5*CA*). Upon incubation at 37°C for 30 min, most of the AZN-D2 Abs disappeared from the plasma membrane (Fig. 5*CB*, arrowheads) and were localized either in small vesicles in proximity of the cell surface or in larger organelles resembling late endosomes (Fig. 5*CB*, arrows). The Abs AZN-D2 (5 nm gold) remained colocalized with DC-SIGN molecules (10 nm gold), indicating that internalized DC-SIGN-associated ligand traffics to endosomal compartments.

We next investigated whether ligand is released from DC-SIGN in compartments with a low pH, enabling the receptor to recycle to

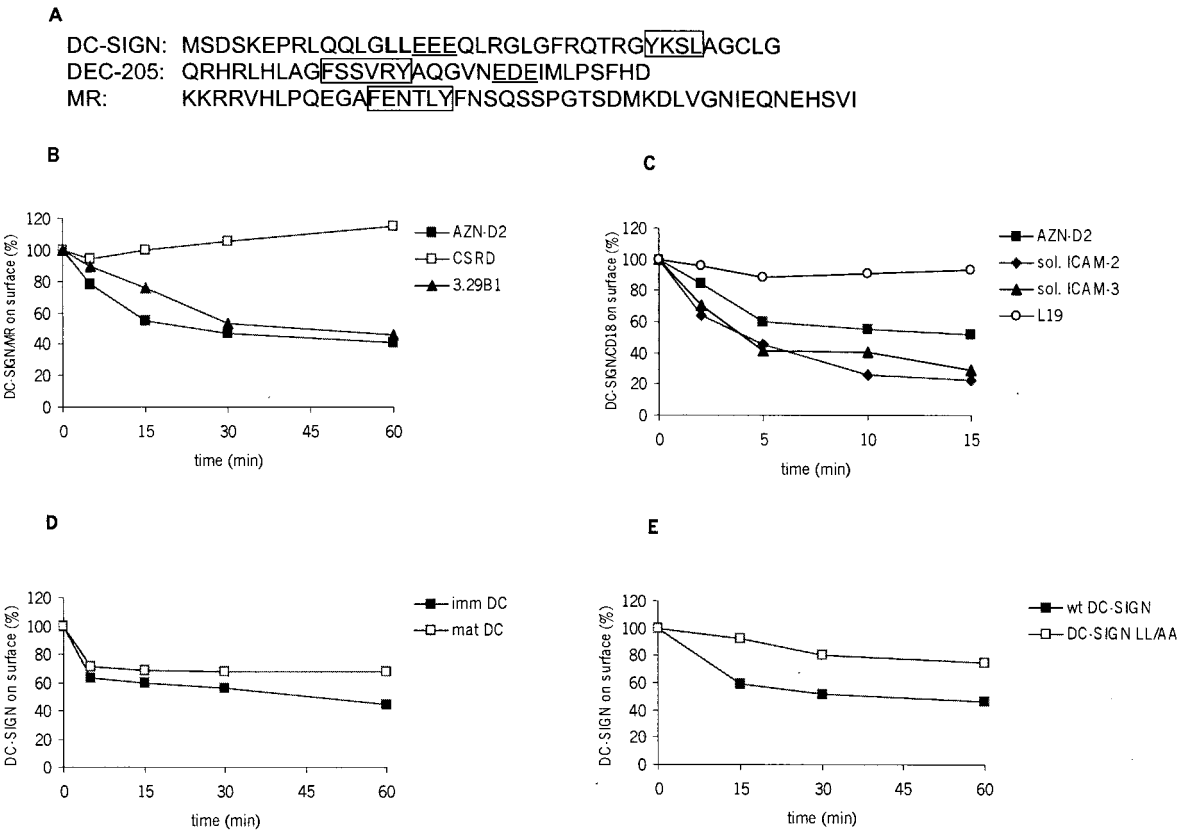

FIGURE 4. Ligand binding induces endocytosis of DC-SIGN that requires the dileucine motif in the cytoplasmic tail. *A*, Amino acid alignment of the cytoplasmic tails from DC-SIGN, DEC-205, and the MR. Putative tyrosine-based internalization motifs are boxed, triacidic motifs are underlined, and the dileucine motif is in bold. *B*, Internalization from the cell surface of bound Abs to the CRD of DC-SIGN (AZN-D2), the C-terminal part of DC-SIGN (CSRD), or the MR (3.29B1) was analyzed on immature DCs using flow cytometry. Fixed cells were used to correct for the off rate of Abs at 37°C. Values represent mean of duplicates. One representative experiment of three is shown. *C*, On immature DCs, internalization from the cell surface of anti-DC-SIGN Abs, soluble ICAM-2- and ICAM-3-Fc, and anti-CD18 (L19) Abs was analyzed as in *B*. *D*, The endocytosis of AZN-D2 was measured on immature and LPS-matured DCs as in *B*. *E*, K562 cells were transfected with wild-type or mutant DC-SIGN, in which two leucine are replaced by alanines, and internalization of AZN-D2 was analyzed as in *B*.

the cell surface, as is the case for the MR and DEC-205. To mimic this, DC-SIGN-mediated binding to HIV-1 gp120 by immature DCs was analyzed at different pH (Fig. 5*D*). At neutral and basic pH, high binding of ligand was observed, whereas at acidic pH, ligand binding was reduced, up to 20% binding at pH 5. This suggests that upon internalization of DC-SIGN-ligand complexes into acidic organelles such as late endosomes and lysosomes, ligand can dissociate, allowing recycling of DC-SIGN to the cell surface.

DC-SIGN captures Ag for presentation to T cells

The targeting of DC-SIGN-ligand complexes to lysosomes and subsequent release suggests processing of the ligand for presentation by MHC molecules. To test this, we made use of a $CD4^+$ T cell clone that recognizes peptides derived from mouse IgG1 Abs (37). Immature DCs from a donor with a compatible haplotype were preincubated with serial dilutions of Abs and their capacity to induce proliferation of T cells was tested. Using the anti-DC-SIGN Abs AZN-D1, AZN-D2 (Fig. 6), and AZN-D3 (data not shown), which are all of IgG1 isotype, specific Ag presentation was induced from concentrations of 10 ng/ml and higher. Isotype control Abs were included as well as an IgG2b anti-DC-SIGN Ab (DCN46.1) and anti-ICAM-2 Abs (IgG1, AZN-ICM2.1). In the presence of these Abs, DCs could not induce T cell proliferation (Fig. 6). Thus, DC-SIGN functions as an efficient Ag receptor on DCs that is endocytosed upon soluble ligand binding.

Discussion

DCs internalize Ags in peripheral tissues, migrate through lymph to the draining lymph node, and efficiently present peptides derived from these Ags to prime naive T lymphocytes. In this study we demonstrate that DC-SIGN, a recently identified DC-specific adhesion receptor, functions as an Ag receptor that can internalize and target Ags to the degradation pathway, resulting in processing and subsequent presentation of these Ags to T cells.

C-type lectins as Ag receptors on DCs

Various pathways exist by which DCs internalize soluble Ags for presentation to T cells, one of which is receptor-mediated endocytosis, in which C-type lectins and FcR play a role (1). C-type lectins that function as Ag receptors contain carbohydrate-binding motifs and facilitate binding and uptake of glycosylated ligands. Most C-type lectins have a high affinity for mannosylated Ags, whereas some have high affinity for galactose (9). Mannosylated proteins are especially abundant in a variety of microorganisms. A growing number of C-type lectins with specificity for mannosylated Ags are found to be expressed by DCs such as the MR and DEC-205, but now also DC-SIGN, which functions as receptor for Ag internalization. Only expression of DC-SIGN, however, is restricted to DCs. The MR has multiple CRDs and has been demonstrated to internalize a variety of glycoproteins from microorganisms by macrophages (38). Therefore, mannose-binding receptors may play an important role in the initiation of immune responses against a

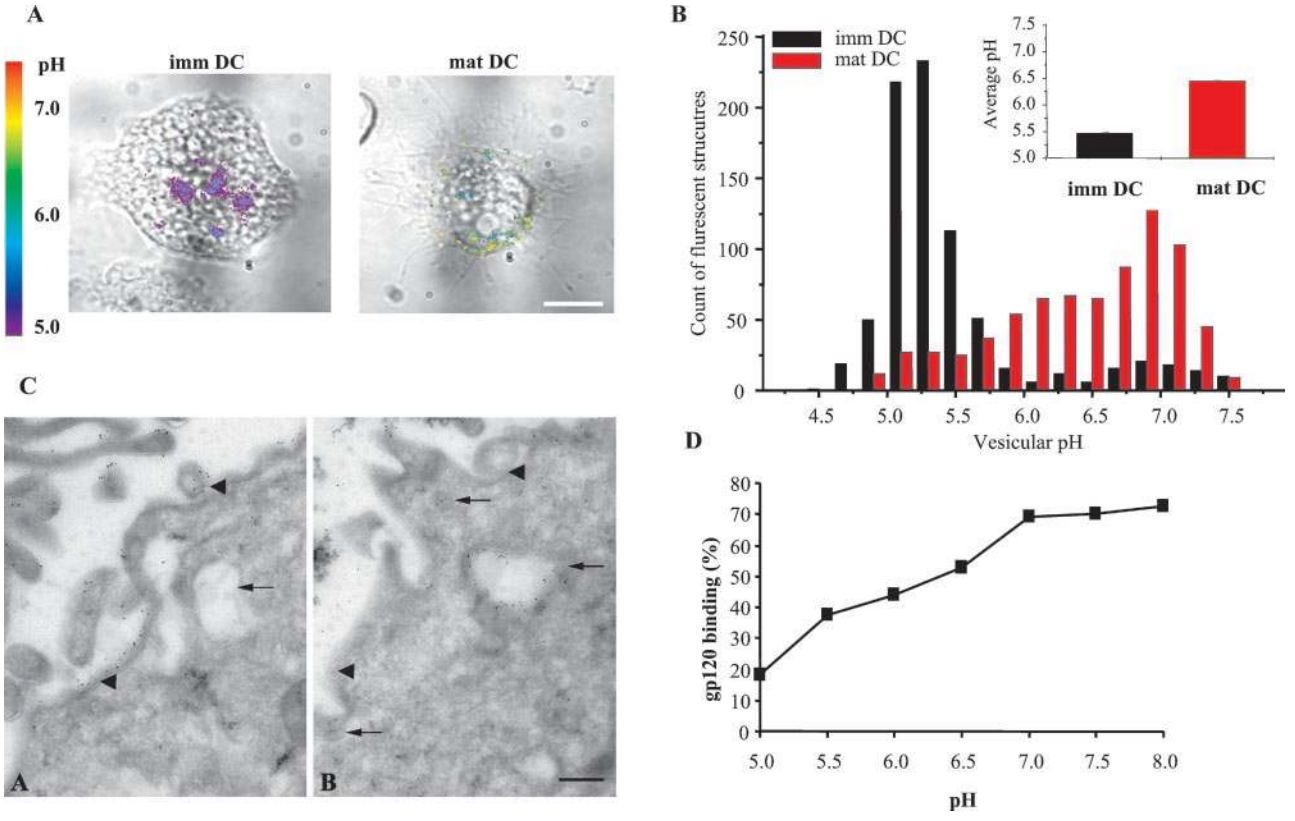

FIGURE 5. Endocytosed DC-SIGN-ligand complexes are targeted to late endosomal/lysosomal compartments. *A* and *B*, Using ratio fluorescence imaging, the pH of internalized AZN-D2 and FITC-conjugated secondary Abs was measured. *A*, Images are shown of a representative immature (*left panel*) and an LPS-matured DC (*right panel*), integrating phase-contrast images and pH, scaled in pseudocolors (pH scale on the *left*). *B*, Distribution of the vesicular pH of internalized DC-SIGN is depicted as analyzed in 804 vesicles in immature DCs and 750 vesicles in mature DCs. *Inset*, Mean pH. Pooled results of three independent experiments are shown. *C*, Subcellular distribution of DC-SIGN-ligand complexes in immature DCs was studied using electron microscopy after binding of AZN-D2 (*CA*) and after triggering internalization at 37°C for 30 min (*CB*). Cryosections were stained with anti-mouse Abs and protein A-5 nm gold to visualize AZN-D2 and subsequently with CSRD and protein A-10 nm gold to detect DC-SIGN. Bar, 200 nm. *D*, pH dependency of gp120-coated fluorescent bead binding was analyzed by flow cytometry after beads binding in buffers of the indicated pH. One representative experiment of three is shown.

diversity of microorganisms. DEC-205 also has recently been reported to function on immature DCs in receptor-mediated endocytosis. DEC-205-ligand complexes are targeted to late endosomes or lysosomes rich in MHC class II products, whereas MR-ligand complexes internalize to more peripheral endosomes. Our results show that DC-SIGN present on immature DCs was rapidly internalized with bound ligand into lysosomal compartments. Subsequently, ligand-derived peptides were presented to T cells in MHC class II, indicating that ligand internalized through DC-SIGN reached MHC class II compartments for processing and peptide loading. It is not clear, however, whether ligand enters these compartments bound to DC-SIGN in immature DCs. On mature DCs, DC-SIGN-mediated internalization of Ag was observed, although at lower efficiency, and was retarded into early endosomal compartments located close to the cell surface, concomitant with the reduced internalization and processing capacity of mature DCs. In our internalization experiments, in which we targeted DC-SIGN with specific Abs or with soluble ligand (ICAM-2- or ICAM-3-Fc), we did not observe any FcR-mediated contribution (Figs. 4 and 6), illustrating that internalization and subsequent processing and presentation of Ag was DC-SIGN mediated.

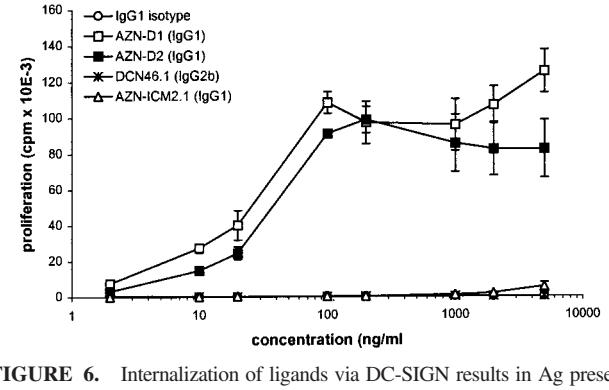

FIGURE 6. Internalization of ligands via DC-SIGN results in Ag presentation to T cells. Proliferation of IgG1-specific T cells induced by immature DCs was analyzed in the presence of serial dilutions of anti-DC-SIGN Abs (AZN-D1 (IgG1), AZN-D2 (IgG1), or DCN 46.1 (IgG2b)), anti-ICAM-2 Abs (AZN-ICM2.1), or IgG1 isotype control Abs. Values represent mean of triplicates ± SD. One representative experiment of two is shown.

The cytoplasmic tail of DC-SIGN contains several motifs that may direct its intracellular targeting (Fig. 4*A*). The tyrosine-based signal of the consensus motif YXXΦ, a recognition site for adapter proteins that dictate intracellular targeting (21), and a dileucine motif involved in targeting to the endosomal/lysosomal pathway (39) are found in the cytoplasmic tail of DC-SIGN. In this study we demonstrate that the dileucine motif supports internalization of DC-SIGN-ligand complexes in transfected cells, because mutation of this motif inhibits DC-SIGN internalization. This is in contrast to the MR and DEC-205, which are constitutively endocytosed through a tyrosine-based motif in the cytoplasmic tail of these molecules (FENTLY and FSSVRY, respectively; Fig. 4*A*) (20). Initial experiments demonstrated that the distinct tyrosine-based

motif in DC-SIGN is not used for internalization and that presumably a constitutively expressed cell surface distribution of DC-SIGN is required for ligand binding and internalization. In addition, the cytoplasmic domain of DC-SIGN contains an acidic triad (EEE) that in DEC-205 mediates targeting into organelles for Ag processing and peptide loading onto MHC class II molecules (20). Indeed, our results indicate that ligands internalized via DC-SIGN are targeted to these compartments, although the exact role of the triacidic motif in the cytoplasmic tail in the intracellular trafficking of DC-SIGN remains to be investigated.

The central role of DCs in the initiation of immune responses has started investigation into exploiting their Ag-presenting capacity in therapeutic strategies against cancer (40). Many naturally occurring tumors such as melanoma are low or nonimmunogenic. The Ag-binding C-type lectins expressed on DCs have been of interest for improving tumor Ag targeting to DCs and enhancing Ag processing and presentation to induce antitumor immunity (17). Recently, it has been demonstrated that DEC-205 is a useful receptor to target DCs in vivo with low amounts of Ags (41). Our findings here demonstrate that DC-SIGN also has the potency to internalize and present Ags. The DC-restricted expression indicates that DC-SIGN can be used to specifically target tumor Ags to DCs, which are essential for inducing an adequate immune response against cancer.

To date, we have demonstrated that Ags internalized and processed through DC-SIGN may lead to MHC class II presentation by using the Ab-specific $CD4^+$ T cell line (Fig. 6). DCs also possess the unique ability to process particulate Ags efficiently into the MHC class I pathway, referred to as cross-presentation (42). Interestingly, it has been recently reported that whole recombinant yeast can activate DCs, which elicit protective cell-mediated antitumor immunity via $CD4^+$ and $CD8^+$ T lymphocytes (43). DC-SIGN binds yeast-derived mannan with a high affinity, indicating that DC-SIGN could be involved in yeast uptake. It is currently unknown whether DC-SIGN also facilitates MHC class I presentation, and future experiments are needed to address these questions.

Ag specificities of C-type lectins

Despite similarities of C-type lectins on DCs and possible redundancy, the specificity for ligands can differ between these lectins. The complexity of mannose structures recognized, the number of mannose groups per ligand, their branching and spacing on the ligand, as well as additional interactions other than carbohydrates may especially differ. Recently, Mitchell et al. (10) demonstrated that DC-SIGN preferentially recognizes high-mannose oligosaccharides. In eukaryotes, cell membrane-bound mannose residues are predominantly present in complex-type N-linked glycoproteins and probably also on viruses, such as HIV. This is in contrast to single terminal-situated mannose residues that are not recognized by DC-SIGN but are bound by mannose-binding protein and the MR. In agreement with these results, we observed that, in contrast to the MR, DC-SIGN does not capture and endocytose mannosylated BSA, which contains single-terminal mannose residues. Also, our finding that DC-SIGN has a much higher affinity for ICAM-3 compared with mannosylated BSA illustrates that whereas DC-SIGN recognizes complex mannose residues in specific arrangements on the surfaces of select glycoproteins, the MR recognizes end-standing single mannoses often present on microorganisms. Moreover, carbohydrate-independent interactions are also important for ligand binding to DC-SIGN (T. Geÿtenbeek, unpublished results). We propose that, instead of being complementary receptors, C-type lectins are functionally distinct on DCs and have distinct recognition profiles to bind specific ligands and pathogens with high affinity. Comparison of distribution of DC-SIGN expression with that of Langerin, DEC-205, and the MR revealed that only DC-SIGN and the MR, which have distinct Ag recognition profiles, are expressed on DCs at the same places in the body, whereas the other C-type lectins are differentially expressed on subsets of DCs.

DC-SIGN functions as an adhesion receptor and an Ag receptor

We have previously shown that DC-SIGN functions as an adhesion receptor on DCs that regulates transendothelial migration of DCs via ICAM-2 and activation of resting T cells through ICAM-3. Here, we show that, as a C-type lectin, DC-SIGN also functions as an Ag receptor on human DCs that can endocytose soluble ligand into lysosomal compartments, resulting in processing of ligand and subsequent presentation to T cells. The dual role of DC-SIGN as adhesion and Ag receptor provides DCs with a functional receptor throughout their life span, i.e., Ag uptake in peripheral tissues, migration across endothelial cells upon inflammation, and subsequent clustering with T lymphocytes upon arrival in lymph nodes. The fact that DC-SIGN functions both as an Ag and as an adhesion receptor indicates that DC-SIGN discriminates itself from the other C-type lectins that so far only function as Ag receptors. Our data indicate that the two different functions of DC-SIGN do not necessarily affect each other, because Ag internalization through DC-SIGN does not affect ICAM-3 binding (data not shown) or T cell proliferation (Fig. 6). The fact that Ag-induced internalization of DC-SIGN does not affect its binding activity might be due to the fact that not all DC-SIGN molecules internalize (Fig. 2) or that the internalized molecules recycle back to the plasma membrane after delivery of Ag in a low-pH environment, similar to the MR and DEC-205 (20, 22). DC-SIGN-mediated internalization experiments demonstrate that, at later time points after internalization of DC-SIGN, expression levels are restored. Whether this rapid reconstitution of DC-SIGN expression after internalization is due to recycling of DC-SIGN or de novo synthesis remains to be determined in future experiments.

Ags that target to DC-SIGN

Until now, the only antigenic ligands known to bind DC-SIGN are lentiviruses, including HIV-1, HIV-2, and SIV-2 (26, 44). We have recently shown that DCs efficiently capture HIV-1 through a high-affinity interaction of gp120 with DC-SIGN and mediate subsequent transmission of HIV-1 to T cells (26). As a result of gp120 binding to DC-SIGN, HIV-1 is stabilized and remains infectious for several days. Here, we show that DC-SIGN is internalized upon soluble ligand binding, suggesting that binding of gp120 to DC-SIGN may result in internalization of virus, facilitating efficient transport to lymph nodes and protection of the virus to promote infection of target T cells (26). However, the question still remains how complete HIV-1 virions can protect themselves against DC-SIGN-mediated internalization and subsequent degradation in low-pH compartments, which would allow Ag processing and presentation for induction of HIV-specific immunity. HIV-1 capture by DCs may interfere either with targeting of DC-SIGN-HIV-1 complexes to lysosomes or with lysosomal degradation. Interestingly, in mature DCs, DC-SIGN targeted to early endosomal compartments in which HIV-1 would be protected against degradation. Recently, we observed that after HIV entry and infection of immature DCs, expression of the HIV-1 protein Nef results in a redistribution of DC-SIGN and an enhanced cell surface expression, thus converting DC-SIGN internalization to cell surface expression and facilitating increased cell adhesion and virus transmission to T cells (45). Further investigation is needed to establish the exact conditions required for HIV to escape degradation and processing by DCs to remain in its infective state for

transmission to T cells or to be processed by DCs to allow anti-HIV immunity.

DCs have the unique capacity to induce cellular responses against a broad variety of Ags. Several C-type lectins on DCs function in capturing Ags. We have shown here that DC-SIGN, an adhesion receptor for ICAM-2 and ICAM-3, can additionally function as Ag receptor. DC-SIGN has the potency to be used for targeting tumor Ags specifically to DCs to induce antitumor immunity. Various C-type lectin receptors on DCs, including DC-SIGN, could be targeted by tumor and viral Ags to determine how DCs can most effectively be used in clinical settings for presentation of tumor/viral Ags and induction of antitumor/viral immunity.

Acknowledgments

We thank Dr. O. Schwartz for the cDNA of the DC-SIGN LL/AA mutant; Drs. M. Cella, M. Colonna, and R. Steinman for Abs; and G. van Duijnhoven and F. Leuba for technical assistance.

References

1. Banchereau, J., and R. M. Steinman. 1998. Dendritic cells and the control of immunity. *Nature 392:245.*
2. Sallusto, F., M. Cella, C. Danieli, and A. Lanzavecchia. 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.*
3. Thery, C., and S. Amigorena. 2001. The cell biology of antigen presentation in dendritic cells. *Curr. Opin. Immunol. 13:45.*
4. Pieters, J. 2000. MHC class II-restricted antigen processing and presentation. *Adv. Immunol. 75:159.*
5. Cella, M., A. Engering, V. Pinet, J. Pieters, and A. Lanzavecchia. 1997. Inflammatory stimuli induce accumulation of MHC class II complexes on dendritic cells. *Nature 388:782.*
6. Pierre, P., S. J. Turley, E. Gatti, M. Hull, J. Meltzer, A. Mirza, K. Inaba, R. M. Steinman, and I. Mellman. 1997. Developmental regulation of MHC class II transport in mouse dendritic cells. *Nature 388:787.*
7. Lanzavecchia, A., and F. Sallusto. 2001. The instructive role of dendritic cells on T cell responses: lineages, plasticity and kinetics. *Curr. Opin. Immunol. 13:291.*
8. Mason, D., ed. 2000. *Leucocyte Typing*, Vol. VII. Oxford University Press, Oxford.
9. Drickamer, K. 1999. C-type lectin-like domains. *Curr. Opin. Struct. Biol. 9:585.*
10. Mitchell, D. A., A. J. Fadden, and K. Drickamer. 2001. A novel mechanism of carbohydrate recognition by the C-type lectins DC-SIGN and DC-SIGNR: subunit organization and binding to multivalent ligands. *J. Biol. Chem. 276:28939.*
11. Valladeau, J., V. Duvert-Frances, J. J. Pin, C. Dezutter-Dambuyant, C. Vincent, C. Massacrier, J. Vincent, K. Yoneda, J. Banchereau, C. Caux, et al. 1999. The monoclonal antibody DCGM4 recognizes Langerin, a protein specific of Langerhans cells, and is rapidly internalized from the cell surface. *Eur. J. Immunol. 29:2695.*
12. Valladeau, J., O. Ravel, C. Dezutter-Dambuyant, K. Moore, M. Kleijmeer, Y. Liu, V. Duvert-Frances, C. Vincent, D. Schmitt, J. Davoust, et al. 2000. Langerin, a novel C-type lectin specific to Langerhans cells, is an endocytic receptor that induces the formation of Birbeck granules. *Immunity 12:71.*
13. Stahl, P. D. 1992. The mannose receptor and other macrophage lectins. *Curr. Biol. 4:49.*
14. Ezekowitz, R. A., K. Sastry, P. Bailly, and A. Warner. 1990. Molecular characterization of the human macrophage mannose receptor: demonstration of multiple carbohydrate recognition-like domains and phagocytosis of yeasts in Cos-1 cells. *J. Exp. Med. 172:1785.*
15. Stahl, P., P. H. Schlesinger, E. Sigardson, J. S. Rodman, and Y. C. Lee. 1980. Receptor-mediated pinocytosis of mannose glycoconjugates by macrophages: characterization and evidence for receptor recycling. *Cell 19:207.*
16. Engering, A. J., M. Cella, D. Fluitsma, M. Brockhaus, E. C. M. Hoefsmit, A. Lanzavecchia, and J. Pieters. 1997. The mannose receptor functions as a high capacity and broad specificity antigen receptor in human dendritic cells. *Eur. J. Immunol. 27:2417.*
17. Tan, M. C., A. M. Mommaas, J. W. Drijfhout, R. Jordens, J. J. Onderwater, D. Verwoerd, A. A. Mulder, A. N. van der Heiden, D. Scheidegger, L. C. Oomen, et al. 1997. Mannose receptor-mediated uptake of antigens strongly enhances HLA class II-restricted antigen presentation by cultured dendritic cells. *Eur. J. Immunol. 27:2426.*
18. Jiang, W., W. J. Swiggard, C. Heufler, M. Peng, A. Mirza, R. M. Steinman, and M. C. Nussenzweig. 1995. The receptor DEC-205 expressed by dendritic cells and thymic epithelial cells is involved in antigen processing. *Nature 375:151.*
19. Kato, M., T. K. Neil, G. J. Clark, C. M. Morris, R. V. Sorg, and D. N. Hart. 1998. cDNA cloning of human DEC-205, a putative antigen-uptake receptor on dendritic cells. *Immunogenetics 47:442.*
20. Mahnke, K., M. Guo, S. Lee, H. Sepulveda, S. L. Swain, M. Nussenzweig, and R. M. Steinman. 2000. The dendritic cell receptor for endocytosis, DEC-205, can recycle and enhance antigen presentation via major histocompatibility complex class II-positive lysosomal compartments. *J. Cell Biol. 151:673.*
21. Bonifacino, J. S., and E. C. Dell'Angelica. 1999. Molecular bases for the recognition of tyrosine-based sorting signals. *J. Cell Biol. 145:923.*
22. Pontow, S. E., V. Kery, and P. D. Stahl. 1992. Mannose receptor. *Int. Rev. Cytol. 137B:221.*
23. Geijtenbeek, T. B., R. Torensma, S. J. van Vliet, G. C. van Duijnhoven, G. J. Adema, Y. van Kooyk, and C. G. Figdor. 2000. Identification of DC-SIGN, a novel dendritic cell-specific ICAM-3 receptor that supports primary immune responses. *Cell 100:575.*
24. Geijtenbeek, T. B., D. J. E. B. Krooshoop, D. A. Bleijs, S. J. van Vliet, G. C. van Duijnhoven, V. Grabosky, R. Alon, C. G. Figdor, and Y. van Kooyk. 2000. DC-SIGN-ICAM-2 interaction mediates dendritic cell trafficking. *Nat. Immun. 1:353.*
25. Curtis, B. M., S. Scharnowske, and A. J. Watson. 1992. Sequence and expression of a membrane-associated C-type lectin that exhibits CD4-independent binding of human immunodeficiency virus envelope glycoprotein gp120. *Proc. Natl. Acad. Sci. USA 89:8356.*
26. Geijtenbeek, T. B., D. S. Kwon, R. Torensma, S. J. van Vliet, G. C. van Duijnhoven, J. Middel, I. L. Cornelissen, H. S. Nottet, V. N. KewalRamani, D. R. Littman, et al. 2000. DC-SIGN, a dendritic cell-specific HIV-1-binding protein that enhances *trans*-infection of T cells. *Cell 100:587.*
27. Blauvelt, A., H. Asada, M. W. Saville, V. Klaus-Kovtun, D. J. Altman, R. Yarchoan, and S. I. Katz. 1997. Productive infection of dendritic cells by HIV-1 and their ability to capture virus are mediated through separate pathways. *J. Clin. Invest. 100:2043.*
28. Bleijs, D. A., M. E. Binnerts, S. J. van Vliet, C. G. Figdor, and Y. van Kooyk. 2000. Low-affinity LFA-1/ICAM-3 interactions augment LFA-1/ICAM-1-mediated T cell adhesion and signaling by redistribution of LFA-1. *J. Cell Sci. 113:391.*
29. Bleijs, D. A., R. de Waal-Malefyt, C. G. Figdor, and Y. van Kooyk. 1999. Costimulation of T cells results in distinct IL-10 and TNF-α cytokine profiles dependent on binding to ICAM-1, ICAM-2 or ICAM-3. *Eur. J. Immunol. 29:2248.*
30. Guo, M., S. Gong, S. Maric, Z. Misulovin, M. Pack, K. Mahnke, M. C. Nussenzweig, and R. M. Steinman. 2000. A monoclonal antibody to the DEC-205 endocytosis receptor on human dendritic cells. *Hum. Immunol. 61:729.*
31. Arrighi, J. F., C. Hauser, B. Chapuis, R. H. Zubler, and V. Kindler. 1999. Long-term culture of human $CD34^+$ progenitors with FLT3-ligand, thrombopoietin, and stem cell factor induces extensive amplification of a $CD34^-CD14^-$ and a $CD34^-CD14^+$ dendritic cell precursor. *Blood 93:2244.*
32. Rescigno, M., V. Piguet, B. Valzasina, S. Lens, R. Zubler, L. French, V. Kindler, J. Tschopp, and P. Ricciardi-Castagnoli. 2000. Fas engagement induces the maturation of dendritic cells (DCs), the release of interleukin (IL)-1β, and the production of interferon γ in the absence of IL-12 during DC-T cell cognate interaction: a new role for Fas ligand in inflammatory responses. *J. Exp. Med. 192:1661.*
33. Fransen, J. A., L. A. Ginsel, H. P. Hauri, E. Sterchi, and J. Blok. 1985. Immunoelectronmicroscopical localization of a microvillus membrane disaccharidase in the human small-intestinal epithelium with monoclonal antibodies. *Eur. J. Cell Biol. 38:6.*
34. Schweizer, A., J. A. Fransen, T. Bachi, L. Ginsel, and H. P. Hauri. 1988. Identification, by a monoclonal antibody, of a 53-kD protein associated with a tubulo-vesicular compartment at the cis-side of the Golgi apparatus. *J. Cell Biol. 107:1643.*
35. Piguet, V., F. Gu, M. Foti, N. Demaurex, J. Gruenberg, J. L. Carpentier, and D. Trono. 1999. Nef-induced CD4 degradation: a diacidic-based motif in Nef functions as a lysosomal targeting signal through the binding of β-COP in endosomes. *Cell 97:63.*
36. Piguet, V., L. Wan, C. Borel, A. Mangasarian, N. Demaurex, G. Thomas, and D. Trono. 2000. HIV-1 Nef protein binds to the cellular protein PACS-1 to downregulate class I major histocompatibility complexes. *Nat. Cell Biol. 2:163.*
37. Lanzavecchia, A., S. Abrignani, D. Scheidegger, R. Obrist, B. Dorken, and G. Moldenhauer. 1988. Antibodies as antigens: the use of mouse monoclonal antibodies to focus human T cells against selected targets. *J. Exp. Med. 167:345.*
38. Fraser, I. P., H. Koziel, and R. A. Ezekowitz. 1998. The serum mannose-binding protein and the macrophage mannose receptor are pattern recognition molecules that link innate and adaptive immunity. *Semin. Immunol. 10:363.*
39. Shen, L., M. L. Lang, and W. F. Wade. 2000. The ins and outs of getting in: structures and signals that enhance BCR or Fc receptor-mediated antigen presentation. *Immunopharmacology 49:227.*
40. Fong, L., and E. G. Engleman. 2000. Dendritic cells in cancer immunotherapy. *Annu. Rev. Immunol. 18:245.*
41. Hawiger, D., K. Inaba, Y. Dorsett, M. Guo, K. Mahnke, M. Rivera, J. V. Ravetch, R. M. Steinman, and M. C. Nussenzweig. 2001. Dendritic cells induce peripheral T cell unresponsiveness under steady state conditions in vivo. *J. Exp. Med. 194:769.*
42. Larsson, M., J. F. Fonteneau, and N. Bhardwaj. 2001. Dendritic cells resurrect antigens from dead cells. *Trends Immunol. 22:141.*
43. Stubbs, A. C., K. S. Martin, C. Coeshott, S. V. Skaates, D. R. Kuritzkes, D. Bellgrau, A. Franzusoff, R. C. Duke, and C. C. Wilson. 2001. Whole recombinant yeast vaccine activates dendritic cells and elicits protective cell-mediated immunity. *Nat. Med. 7:625.*
44. Pohlmann, S., F. Baribaud, B. Lee, G. J. Leslie, M. D. Sanchez, K. Hiebenthal-Millow, J. Munch, F. Kirchhoff, and R. W. Doms. 2001. DC-SIGN interactions with human immunodeficiency virus type 1 and 2 and simian immunodeficiency virus. *J. Virol. 75:4664.*
45. Sol-Foulon, N., A. Moris, C. Nobile, C. Boccaccio, A. Engering, J. Abastado, J. Heard, Y. van Kooyk, and O. Schwartz. 2002. HIV-1 Nef-induced up-regulation of DC-SIGN in dendritic cells promotes lymphocyte clustering and viral spread. *Immunity 16:1.*