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Identification of DC-SIGN, a Novel Dendritic Cell–Specific ICAM-3 Receptor that Supports Primary Immune Responses

Teunis B. H. Geijtenbeek, Ruurd Torensma, Sandra J. van Vliet, Gerard C. F. van Duijnhoven, Gosse J. Adema, Yvette van Kooyk,* and Carl G. Figdor
Department of Tumor Immunology
University Medical Center St. Radboud
Philips van Leydenlaan 25
Nijmegen 6525 EX
The Netherlands

Summary

Contact between dendritic cells (DC) and resting T cells is essential to initiate a primary immune response. Here, we demonstrate that ICAM-3 expressed by resting T cells is important in this first contact with DC. We discovered that instead of the common ICAM-3 receptors LFA-1 and αDβ2, a novel DC-specific C-type lectin, DC-SIGN, binds ICAM-3 with high affinity. DC-SIGN, which is abundantly expressed by DC both in vitro and in vivo, mediates transient adhesion with T cells. Since antibodies against DC-SIGN inhibit DC-induced proliferation of resting T cells, our findings predict that DC-SIGN enables T cell receptor engagement by stabilization of the DC–T cell contact zone.

Introduction

Dendritic cells (DC) are professional antigen-presenting cells that efficiently capture antigens in the peripheral tissues and process these antigens to form MHC–peptide complexes. After antigen uptake, these immature DC acquire the unique capacity to migrate from the periphery to the T cell areas of secondary lymphoid organs. As the cells travel, they mature and alter their profile of cell surface molecules, to attract resting T cells and present their antigenic load (Shaw et al., 1986; Adema et al., 1997; Banchereau and Steinman, 1998).

How the initial contact between DC and resting T cells, necessary for T cell activation, is established and regulated is still largely unknown. Early studies indicated that DC and T cells tend to form clusters in an antigen-independent manner (Steinman and Cohn, 1974). Both adhesion and costimulatory molecules with their corresponding ligands, such as LFA-3/CD2 and LFA-1/ICAM-1, -2 or -3, are potential candidates to mediate these DC–T cell interactions (Springer et al., 1987; Hauss et al., 1995; Starling et al., 1995). Several studies have suggested that binding of LFA-1 to ICAM-3, which is expressed at high levels on resting T cells, might be important in establishing these initial DC–T cell interactions (Vilella et al., 1990; de Fougerolles and Springer, 1992; Hauss et al., 1995; Starling et al., 1995). Separate from mediating adhesion between DC and T cells, ICAM-3 may be important in providing signals necessary for efficient T cell activation (Hernandez-Caselles et al., 1993). Indeed, antibody engagement of ICAM-3 results in elevation of intracellular calcium levels and induction of tyrosine phosphorylation (Juan et al., 1994).

The β2 integrins LFA-1 (αLβ2) and αDβ2 have both been described as the primary receptors for ICAM-3, although the affinity of both integrins for ICAM-3, even after integrin activation (Dustin and Springer, 1989; van Kooyk et al., 1989), is rather low (Binnerts et al., 1994; Vandervieren et al., 1995). Because LFA-1 is expressed by DC and anti-LFA-1 antibodies partially inhibit DC–T cell interactions (Hauss et al., 1995; Starling et al., 1995), it potentially mediates binding of resting T cells through ICAM-3. However, the multitude of adhesion receptors and ligands that simultaneously contribute to the DC–T cell contacts form a major obstacle in antibody blocking studies to dissect the individual role of each adhesion receptor. To circumvent this problem, we developed an adhesion assay (Geijtenbeek et al., 1999) in which fluorescent beads coated with ICAM-3 allow us to specifically address the role of ICAM-3 in DC–T cell interactions. Here, we report that neither LFA-1 nor αDβ2 but a novel ICAM-3 binding C-type lectin, exclusively expressed by DC, mediates strong adhesion between DC to ICAM-3 on resting T cells and is essential for DC-induced T cell proliferation.

Results

Adhesion of DC to ICAM-3 Is Mediated by DC-SIGN

The extremely high expression of ICAM-3 compared to ICAM-1 on resting T cells led us to hypothesize that ICAM-3-mediated adhesion might be crucial in DC–T cell interactions. Exploiting a recently developed flow cytometric adhesion assay (Geijtenbeek et al., 1999), we tested the capacity of immature DC and monocytes to bind to fluorescent beads coated with ICAM-3-Fc. DC strongly bind to ICAM-3 when compared to monocytes (Figure 1A). Whereas ICAM-3 binding by monocytes is for the greater part LFA-1 mediated, since both anti-LFA-1 (αL) and anti-β2 integrin antibodies significantly inhibit adhesion of monocytes to ICAM-3, binding of ICAM-3 by DC is completely LFA-1 independent (Figure 1A). This integrin-independent binding is further substantiated by the findings that neither antibodies directed against the other β2 integrins (αD, αM, αX, data not shown) nor antibodies directed against β subunits of other integrins (β1, β2, β3, β4, and β7, Figure 1B; data not shown) can inhibit ICAM-3 binding by DC. Interestingly, though the interaction is integrin independent it requires the presence of Ca^{2+}, as both EDTA and EGTA are inhibitory (Figure 1B). The specificity of this adhesion receptor on DC for ICAM-3 is demonstrated by the ability of the antibodies against ICAM-3 to inhibit binding (Figure 1B). From these observations, we concluded that DC interact with ICAM-3 through an integrin-independent mechanism that requires Ca^{2+}. We designated this receptor DC-specific ICAM-3 grabbing nonintegrin (DC-SIGN).

*To whom correspondence should be addressed (e-mail: y.vankooyk@dent.kun.nl).

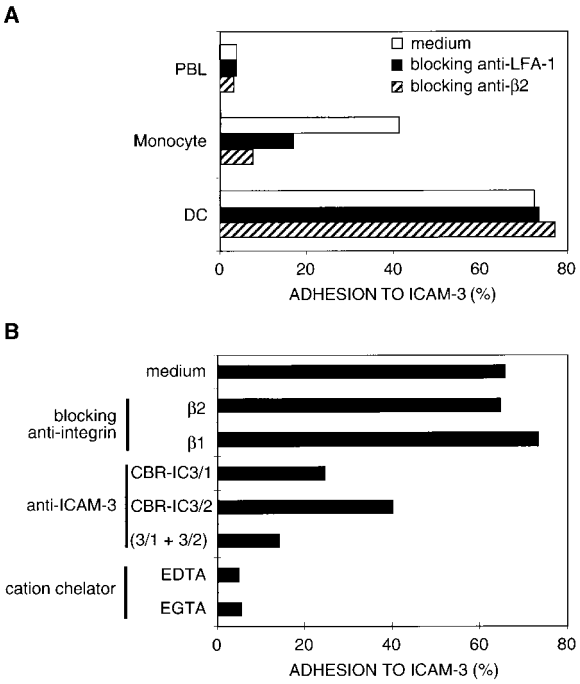


Figure 1. Adhesion of Immature DC to ICAM-3 Is Ca^{2+} Dependent and Integrin Independent

(A) Spontaneous adhesion of peripheral blood leukocytes (PBL), monocytes, and immature DC to ICAM-3. LFA-1- and β2 integrin-specific adhesion was determined in the presence of blocking mAb (20 μg/ml) against LFA-1 (NKI-L15) and β2 integrins (AZN-L19).

(B) Adhesion of immature DC to ICAM-3 in the presence of blocking mAb (20 μg/ml) against β2 integrins (β2 chain, AZN-L19), β1 integrins (β1 chain, AIIB2), and ICAM-3 (CBR-IC3/1, CBR-IC3/2) and in the presence of EDTA (5 mM) or EGTA (5 mM). In (A) and (B), one representative experiment out of three is shown (SD $<$ 5%).

DC-SIGN Is a 44 kDa Protein Identical to the HIV-1 gp120 Binding C-Type Lectin

To characterize DC-SIGN in more detail, we raised monoclonal antibodies against this novel ICAM-3 adhesion receptor. Two hybridomas, AZN-D1 and AZN-D2, were isolated. Antibodies secreted by both hybridomas strongly inhibit adhesion of DC to ICAM-3 (Figure 2A) This, together with the observation that both antibodies do not affect LFA-1-mediated adhesion of monocytes to ICAM-3, suggests that these antibodies are specific for DC-SIGN (Figure 2A).

SDS-PAGE analysis of immunoprecipitated DC-SIGN from a ^{125}I-surface-labeled DC lysate revealed that DC-SIGN is a single protein of 44 kDa (Figure 2B). After excision of this 44 kDa band from the SDS-PAGE gel, the amino acid sequence of two peptides (0.5–1 pmol) was deduced (Edman degradation procedure). The peptides consisted of 12 and 14 amino acid residues, respectively, and 11 amino acid residues of both peptides were identified (Figure 2C). Both peptide sequences proved 100% identical to the deduced amino acid sequence of the HIV-1 envelope glycoprotein gp120 binding C-type lectin (Curtis et al., 1992). This protein was cloned from a human placenta cDNA library and reported to be a CD4-independent receptor for binding of the HIV-1 envelope glycoprotein gp120. The cells that expressed this protein in human placenta were not identified (Curtis et al., 1992).

RT-PCR analysis of RNA isolated from DC with primers based on the gp120-binding C-type lectin sequence yielded a PCR product of the expected length of 1237 nucleotides. Subsequent nucleotide sequence analysis confirmed that DC-SIGN is identical to the HIV-1 gp120 binding C-type lectin (data not shown). DC-SIGN is a type II transmembrane protein consisting of 404 amino acids with three distinct domains. The N-terminal cytoplasmic domain of 40 amino acid residues is separated by a hydrophobic stretch of 15 amino acids from a region that consists of seven complete and one incomplete tandem repeat of nearly identical sequence. The extracellular C-terminal region (Cys253-Ala404) shows homology to Ca^{2+}-dependent (C-type) lectins (Figure 2C).

To prove that DC-SIGN is indeed an ICAM-3 receptor, we transiently transfected COS7 cells with cDNA encoding DC-SIGN. Flow cytometric analysis of these transfectants unequivocally proves that this cDNA encodes for DC-SIGN (Figure 2D, inset). About 30% of the COS7 cells stained with anti-DC-SIGN antibody and therefore expressed DC-SIGN. Moreover, the COS7 cells expressing DC-SIGN strongly bind ICAM-3, whereas mock transfected COS7 cells do not (Figure 2D). As expected, binding of ICAM-3-coated beads by COS7-DC-SIGN cells is Ca^{2+} dependent and can be inhibited by antibodies against ICAM-3 or DC-SIGN (Figure 2D). Similar results were obtained with hematopoietic cells when transfected with the DC-SIGN cDNA. Stable expression of DC-SIGN by the erythroleukemic cell line K562 as well as the promonocytic cell line THP-1 resulted in strong binding of ICAM-3 (data not shown).

Since both DC-SIGN and LFA-1 bind ICAM-3, we studied their relative binding affinity for soluble ICAM-3 (Figure 3A) (Yauch et al., 1997; van Kooyk et al., 1999). DC-SIGN strongly binds soluble ICAM-3 with an IC50 of 7 μg/ml ICAM-3Fc. In contrast, LFA-1 is not active on DC and therefore does not bind ICAM-3 (Figure 1), even at high concentrations of ICAM-3 (Figure 3A). This cannot be attributed to low expression levels of LFA-1 on DC since HSB T cells, which express similar levels of an active form of LFA-1 (Binnerts and van Kooyk, 1999), readily bind ICAM-3 (Figure 3A, IC50 = 15 μg/ml).

As DC-SIGN is a C-type mannose-binding lectin (Curtis et al., 1992; Weis et al., 1998) containing a CRD motif that binds two Ca^{2+} ions and mannose (Drickamer, 1995), we investigated whether Ca^{2+} cations and carbohydrates contribute to the recognition of ICAM-3 by DC-SIGN. Titration of Ca^{2+} concentrations demonstrated that the concentration around 0.3 mM Ca^{2+} gave maximal ICAM-3 binding (second order Calcium binding) in accordance to other C-type lectins (Figure 3B) (Mullin et al., 1997). Other bivalent cations were not capable of mediating ligand binding. Inhibition studies using series of saccharides demonstrated that mannan is the most potent carbohydrate inhibitor (Figure 3C) (IC50 = 1 mM) and blocks ICAM-3 binding as efficiently as anti-DC-SIGN antibodies. Similarly, D(+)-mannose inhibits ICAM-3 adhesion although with lower affinity than mannan. These data suggest that ICAM-3 binding by DC-SIGN depends on binding of two Ca^{2+} and is at least partially dependent on mannose moieties.

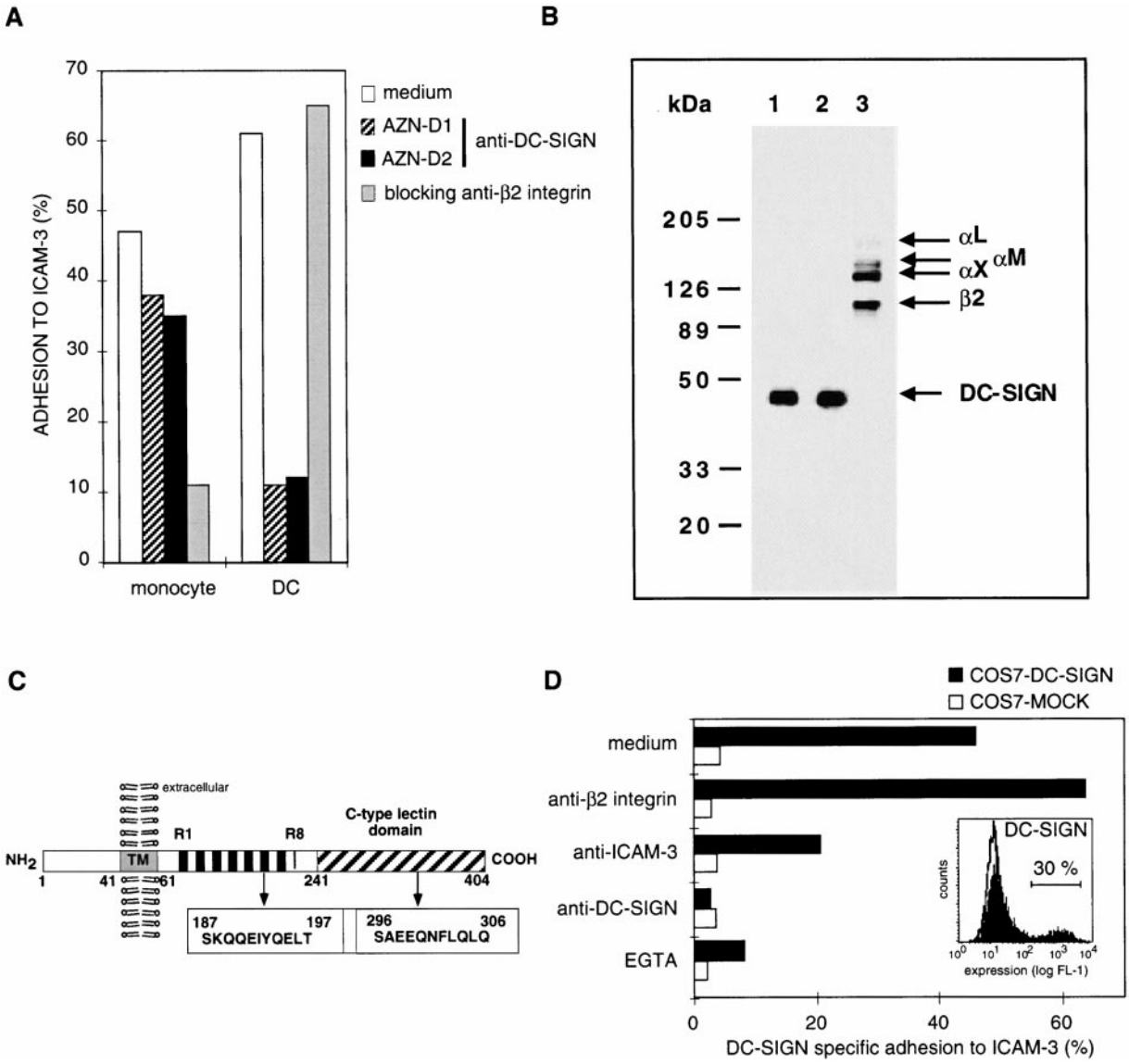


Figure 2. Molecular Characterization of DC-SIGN

(A) Anti-DC-SIGN mAb AZN-D1 and AZN-D2 (20 μg/ml) block adhesion of immature DC but not that of freshly isolated monocytes to ICAM-3Fc-coated fluorescent beads. Adhesion of monocytes to ICAM-3 is blocked by anti-β2 integrin mAb (AZN-L19, 20 μg/ml). A representative experiment out of three experiments is shown (SD $<$ 5%).

(B) DC-SIGN is a 44 kDa protein. DC were surface labeled with I^{125} and lysed, and DC-SIGN was immunoprecipitated with anti-DC-SIGN antibodies AZN-D1 (lane 1) and AZN-D2 (lane 2). The β2 integrin chain and the α chains (αL, αM, and αX) were immunoprecipitated with AZN-L19 (anti-β2 integrin; lane 3). The immunoprecipitates were analyzed by SDS-PAGE (5%–15% gel, reduced conditions) followed by autoradiography. The migration of the molecular weight markers is indicated on the left. The arrows indicate the α chains of LFA-1 (αL, 180 kDa), MAC-1 (αM, 165 kDa) and p150,95 (αX, 150 kDa), the β2 integrin chain (95 kDa), and DC-SIGN (44 kDa). Similar results were obtained in three other experiments.

(C) Schematic presentation of DC-SIGN isolated from human DC. Immature DC were lysed (1% Nonidet-40), and analysis of the immunoprecipitates, performed with anti-DC-SIGN mAb, revealed a protein of 44 kDa. After immunoprecipitation (B), the protein was excised from the gel, and tryptic peptides were sequenced. The two boxed peptide sequences were identified (corresponding to amino acid positions 187–197 and 296–306 of the human placenta gp120 binding C-type lectin [Curtis et al., 1992]). The nucleotide sequence encoding DC-SIGN is 100% identical to that of the human placenta gp120 binding C-type lectin. The transmembrane region (TM), the C-type lectin domain, and the seven complete and one partial repeat (R1-R8) are indicated.

(D) Adhesion of COS7 cells, transiently transfected with DC-SIGN, to ICAM3. Anti-DC-SIGN antibodies (AZN-D1) recognize COS7 cells transfected with the cDNA encoding DC-SIGN (filled histogram, 30% positive cells as determined by flow cytometry, see inset). Mock transfected cells (open histogram, see inset) are not stained. Adhesion of COS7DC-SIGN cells to ICAM-3 was measured in the presence of EGTA (5 mM) and blocking antibodies against DC-SIGN (AZN-D1), ICAM-3 (CBR-IC3/1, CBR-IC3/2), or β2 integrins (AZN-L19). Adhesion was determined as described in Figure 1. A representative experiment out of three is shown (SD $<$ 5%).

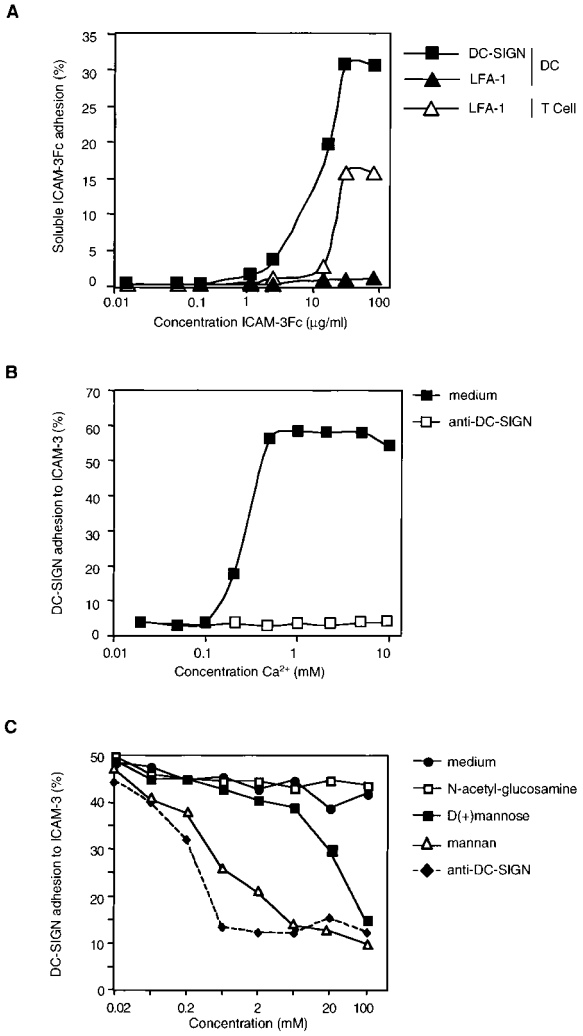


Figure 3. DC-SIGN-Mediated ICAM-3 Binding

(A) DC-SIGN binds ICAM-3 with higher affinity than LFA-1. Adhesion of soluble ICAM-3Fc was measured to both immature DC and HSB T cells. LFA-1 on the HSB T cells has been activated with anti-β2 integrin antibody KIM185. Binding of ICAM-3Fc (30 min room temperature) was analyzed by flow cytometry after staining with a FITC-conjugated anti-human Fc antibody. To determine the receptors involved in ICAM-3Fc binding, both anti-DC-SIGN and anti-LFA-1 antibodies were used to block adhesion, and the DC-SIGN and LFA-1-specific adhesion is depicted. A representative experiment out of three is shown (SD $<$ 5%).

(B) DC-SIGN-mediated ICAM-3 binding is Ca^{2+} dependent. Binding of DC to ICAM-3Fc-coated beads is measured at different Ca^{2+} concentrations in the presence and absence of anti-DC-SIGN antibodies (AZN-D1, 20 μg/ml). The resulting curve best fitted to the equation for second order dependence to Ca^{2+} (fractional binding) = $[Ca^{2+}]^2$ / $((KCa^{2+})^2 + [Ca^{2+}]^2)$, which demonstrates that DC-SIGN binds two Calcium ions similarly as has been shown for other C-type lectins (Mullin et al., 1997). A representative experiment out of three is shown.

(C) DC-SIGN-mediated ICAM-3 binding is mediated by carbohydrates. Different carbohydrates were used to inhibit the adhesion of DC to ICAM-3Fc beads. As shown, DC-SIGN belongs to the C-type lectins that can bind mannose-like carbohydrates (Drickamer, 1999). A representative experiment out of three is shown.

DC-SIGN Expression Is DC Specific

Differentiation of monocytes into immature DC in vitro in the presence of GM-CSF and IL-4 coincides with DC-SIGN expression at extremely high levels (Figure 4A). Expression is already observed from day 1 onward (Figure 4A). Expression gradually increases in time, in contrast to the expression of LFA-1, which remains unaltered (Figure 4A). In accordance with literature (Sallusto and Lanzavecchia, 1994), expression of the monocyte marker CD14 decreases during culture, and at day 7 only low CD14 expression is observed on DC (Figure 4A). Further phenotypic analyses demonstrate that at day 7 the cells express high levels of ICAM-1, αMβ2, αXβ2, MHC class I and II, and moderate levels of CD86 (data not shown), consistent with the phenotype of immature DC (Romani et al., 1994; Sallusto and Lanzavecchia, 1994). Further maturation of DC in the presence of cytokines (TNF, IL-1, or LPS) did not significantly enhance DC-SIGN expression (data not shown). Besides monocyte-derived DC, DC-SIGN is also expressed by DC obtained from other DC precursors such as bone marrow $CD34^+$ cells (data not shown). Together, these results demonstrate that DC generated in vitro abundantly express DC-SIGN at day 7 and that expression of DC-SIGN is several fold higher than that of LFA-1. Also, the expression level of DC-SIGN correlates with DC-SIGN-mediated adhesion of DC to ICAM-3 (Figure 4B). Further flow cytometric analysis of an extensive panel of hematopoietic cells with anti-DC-SIGN antibodies demonstrates that DC-SIGN is exclusively expressed by both immature and mature DC (Table 1). DC-SIGN is not expressed by monocytes, activated monocytes, monocytic cell lines, granulocytes, T cells, B cells, activated B and T cells, thymocytes, and $CD34^+$ bone marrow cells.

To examine whether DC-SIGN expression in vitro coincides with expression in situ, sections of lymph node, spleen, tonsil, and skin were stained with anti-DC-SIGN antibodies (Figure 5). DC-SIGN-expressing cells are present in the T cell area of both lymph nodes (a and b), tonsils (c), and spleen (d). These cells are large and very irregular in shape, consistent with the distribution and morphology of DC (Figure 5b). No staining of DC-SIGN is observed in germinal centers and mantle zones of the lymphoid tissues (Figures 5a–5d). Analyses of serial sections stained for CD3, CD20, CD14, and CD68 confirm that the DC-SIGN-expressing cells are distinct from T cells, B cells, monocytes, and macrophages, respectively (data not shown). In skin sections, DC-SIGN is only expressed on dermal DC (Figure 5e), whereas CD1a positive Langerhans cells in the epidermis are negative (Figure 5f). Furthermore, DC-SIGN is expressed on DC-like cells present in the mucosal tissues, such as rectum, cervix, and uterus (Geijtenbeek et al., 2000 [this issue of *Cell*]) and are localized underneath the stratified squamous epithelium in the lamina propria. From these findings, we conclude that DC-SIGN is specifically expressed by DC both in vitro and in vivo.

DC-SIGN Mediates DC–T Cell Clustering and DC-Induced Proliferation of Resting T Cells

A major obstacle in identifying the molecules that participate in DC–T cell interactions is caused by the multitude

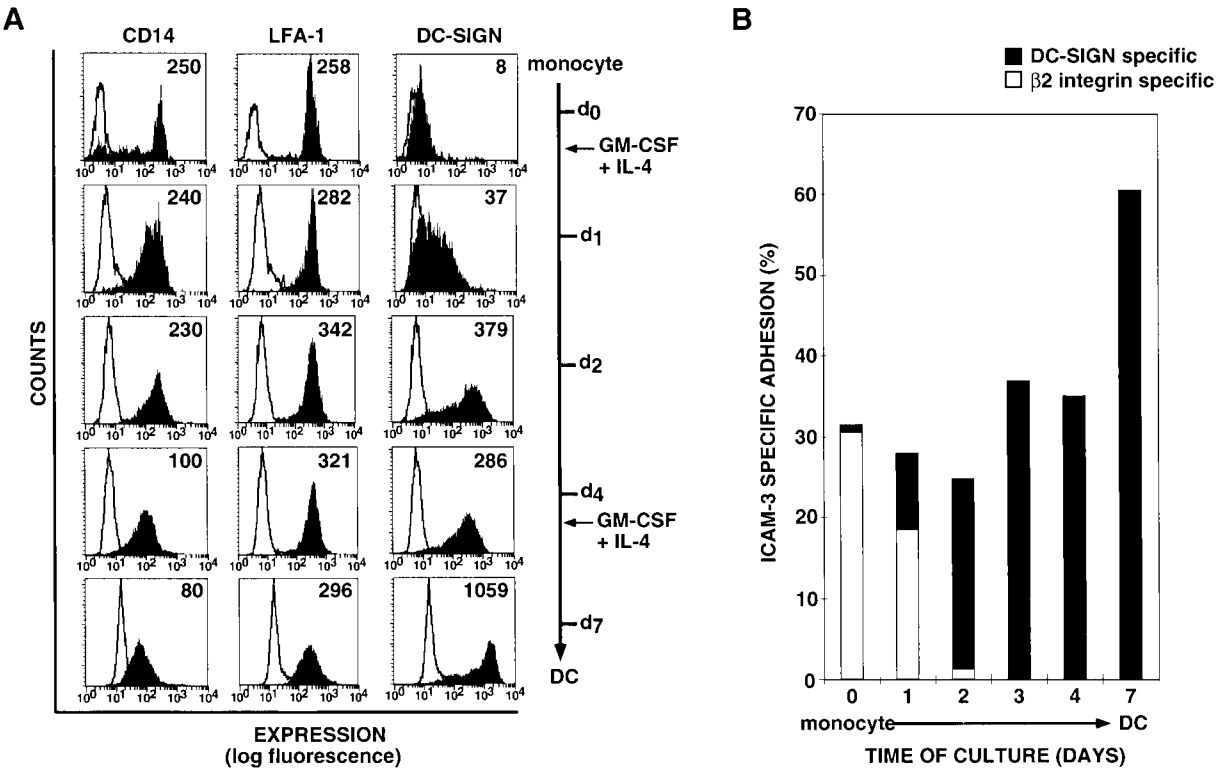


Figure 4. Binding of ICAM-3 Shifts from Integrin Mediated to DC-SIGN Mediated during Development of Monocyte-Derived DC In Vitro

(A) DC-SIGN expression increases during DC development in vitro. Immature DC were cultured from monocytes in the presence of GM-CSF and IL-4. At different time points, the developing DC were analyzed for expression of the monocyte marker CD14, the β2 integrin LFA-1 (NKI-L15), and DC-SIGN (AZN-D1). Cells were gated on forward-side scatter, and the mean fluorescence is shown in the top right corner of the histograms. One representative experiment out of three is shown.

(B) Immature DC developing from monocytes increasingly bind to ICAM-3 in a DC-SIGN-dependent manner. At different time points during culture, the ICAM-3-specific adhesion was determined in the presence of blocking anti-β2 integrin (AZN-L19) or anti-DC-SIGN (AZN-D1) mAb (20 μg/ml). Specific adhesion of DC-SIGN and the β2 integrins is calculated from the inhibition of adhesion in the presence of the blocking mAb AZN-D1 or AZN-L19, respectively. A representative experiment out of three is shown (SD < 5%).

of interactions that contribute simultaneously. In an attempt to evaluate the role of DC-SIGN in this process, we analyzed the capacity of DC to cluster with ICAM-3 transfectants (K562-ICAM-3). For this purpose, we chose K562 cells that lack expression of β1 and β2 integrins (except α5β1) and only express ICAM-1. The results in Figure 6A show that DC form clusters with K562-ICAM-3 in a DC-SIGN-dependent manner since anti-DC-SIGN antibodies block the interaction. Interestingly, DC-SIGN-dependent clustering is transient, indicating that DC-SIGN/ICAM-3 interactions are actively regulated. The gradual increase in DC-SIGN-independent clustering of DC to untransfected K562 cells is caused by LFA-1 on DC that binds ICAM-1 on K562 cells (data not shown).

To demonstrate that DC-SIGN/ICAM-3 interactions are also essential in DC–T cell binding and T cell activation, we analyzed the involvement of DC-SIGN in both DC–T cell clustering and DC-induced T cell proliferation. DC cluster efficiently with allogeneic resting T cells reaching a maximum at 25 min after initial contact (Figure 6B). The transient nature of this DC–T cell clustering is not completely DC-SIGN dependent, since also other adhesion molecules, such as LFA-1 and LFA-3, contribute in stabilizing DC–T cell contact. DC-SIGN-mediated binding to ICAM-3 reaches a maximum at 19 min after DC–T cell contacts (Figure 6B). Also, LFA-1- and LFA-3-mediated DC–T cell clustering is transient and followed the kinetics of the DC-SIGN-mediated adhesion, reaching a maximum at 23 min after initial DC–T cell contact (Figure 6B).

To examine if these DC-SIGN-mediated interactions are important in the initiation of primary immune responses, we tested the capacity of allogeneic DC to induce proliferation of resting T cells compared to activated T cells. As demonstrated in Figure 6C, the presence of anti-DC-SIGN antibodies inhibit the DC-induced proliferation of resting T cells for more than 60%. Similarly to DC–T cell clustering, also here LFA-1 and LFA-3 contribute to the DC-induced T cell proliferation, since antibodies against β2 integrins (LFA-1) and LFA-3 affect proliferation as well (Figure 6C). Antibodies against ICAM-1 inhibited proliferation comparable to antibodies against β2 integrins whereas anti-ICAM-3 antibodies do not inhibit proliferation (data not shown), which can be ascribed to the costimulatory effects of anti-ICAM-3 antibodies in T cell proliferation as was observed previously (Delpozo et al., 1994). When anti-LFA-3 and anti-DC-SIGN antibodies are combined, a complete block of DC-induced T cell proliferation is observed (Figure 6C).

Table 1. Expression Level of DC-SIGN on Hematopoietic Cells as Determined by Flow Cytometric Analyses and RT-PCR

Cell Type	DC-SIGN Expression[a]	DC-SIGN mRNA[b]
Monocytes	≤15	−
Immature DC day 7	980	+
Mature DC day 9[c]		
LPS	489	+
MCM/PGE2	445	+
TNFα	420	+
PBL	≤15	−
T cells	≤15	−
B cells	≤15	−
Thymocytes	≤15	−
Granulocytes	≤15	−
$CD34^+$ cells	≤15	ND
PBMC (activated[d])	≤15	−
T cell lines[e]	≤15	−
Monocytic cell lines[f]	≤15	−

[a] Mean fluorescence of staining with AZN-D1.
[b] RT-PCR with the DC-SIGN-specific primers XF29 and XR1265 on total RNA isolated from the different cells.
[c] Immature DC were maturated with GM-CSF, IL-4 and either TNFα, LPS or a combination of Monocyte Conditioned Medium (MCM) and prostaglandin E2.
[d] Activated with PHA (10 (g/ml) and IL-2 (10 U/ml) for 2 days.
[e] T cell lines: HSB, PEER, CEM and Jurkat.
[f] Monocytic cell lines: THP-1, MM6, and U937.
ND, not determined.

In contrast, combinations of anti-DC-SIGN antibodies with antibodies against ICAM-1, ICAM-3, or LFA-1 do not further reduce the T cell proliferation when compared with anti-DC-SIGN antibodies alone (data not shown).

The observation that resting T cells express high levels of ICAM-3 and low levels of ICAM-1, compared to activated T cells that express equal amounts of ICAM-3 and ICAM-1 (de Fougerolles and Springer, 1992; Hauss et al., 1995; Starling et al., 1995), suggests that DC-SIGN/ICAM-3 interactions are of particular importance in the activation of resting T cells rather than maintaining responses of previously activated T cells. This notion was supported by the finding that neither anti-DC-SIGN antibodies nor ICAM-3 antibodies (Figure 6D) or a combination thereof (data not shown) was capable of inhibiting T cell stimulation of activated T cells. These findings demonstrate that DC-induced proliferation of activated T cells is mainly mediated by LFA-3/CD2 with little contribution of DC-SIGN.

To exclude the possibility that the effects observed with anti-DC-SIGN antibodies are caused by altered expression of other important surface molecules on DC such as LFA-1, LFA-3, and MHC class I and II that affect cell–cell binding, we analyzed the DC phenotype before and after exposure to anti-DC-SIGN. No changes were observed in either of the molecules tested (Figure 6E). Similarly, expression of Mac-1, p150,95, and CD4 is not affected by anti-DC-SIGN antibodies (data not shown).

Discussion

DC are the strongest antigen-presenting cells and function as sentinels of the immune system (Steinman, 1991; Banchereau and Steinman, 1998). They possess a superior antigen processing and presentation machinery. In addition, they are equipped with unique migratory capacities allowing these cells to capture antigen in the periphery and present these to resting/naive T cells that reside in the lymphoid organs. Seminal observations made in the early seventies (Steinman and Cohn, 1973; Steinman et al., 1974), showing rosettes of T cells around DC, already hinted at the importance of adhesion receptors on DC to facilitate T cell contact.

DC-SIGN Is a Dendritic Cell–Specific Adhesion Receptor

Here, we have identified a novel DC-specific C-type lectin, DC-SIGN, that mediates the initial contact of DC with resting T cells. Therefore, the staggering antigen-presenting capacity of DC may now—in addition to their unique antigen processing machinery—at least in part be attributed to their ability to interact with resting T cells through DC-SIGN. Our results demonstrate that DC-SIGN is a major ICAM-3 receptor on DC and essential in establishing the initial transient DC–T cell contact. This is supported by the high relative binding affinity of DC-SIGN for ICAM-3 when compared to that of LFA-1 (Figure 3A). Therefore, this C-type lectin, rather than β2 integrins, is the predominant ICAM-3 receptor on DC. Binding of ICAM-3 by DC-SIGN is Ca^{2+} dependent and can be inhibited by mannose, which is characteristic for C-type lectins that contain Ca^{2+}-dependent extracellular carbohydrate recognition domains (CRDs). DC express other C-type lectins, such as the macrophage-mannose receptor (MMR) and DEC-205, that bind and internalize carbohydrate-bearing antigens by receptor-mediated endocytosis (Jiang et al., 1995; Sallusto et al., 1995). The finding that C-type lectins are involved in the uptake and presentation of antigens via DC (Jiang et al., 1995), and the fact that mannosylation of antigen leads to a selective targeting and superior presentation by DC (Tan et al., 1997), indicates that DC-SIGN as a C-type lectin may also have an important consequences for antigen presentation by DC.

Peptide amino acid sequencing demonstrated that DC-SIGN is identical to the HIV-1 envelope glycoprotein g120 binding C-type lectin cloned from a placental cDNA library (Curtis et al., 1992). In the accompanying paper (Geijtenbeek et al., 2000), we demonstrate that indeed DC-SIGN expressed by DC also binds the HIV-1 envelope glycoprotein gp120. DC, present in initial sites of HIV-1 infection, efficiently capture HIV-1 through DC-SIGN and facilitate the infection of T cells through a unique in *trans* mechanism (Geijtenbeek et al., 2000). Both gp120 (Curtis et al., 1992; data not shown) and ICAM-3 (Figures 3B and 3C) are bound by DC-SIGN in a Ca^{2+}-dependent mechanism (Figure 3B), and binding can be inhibited by mannan (Figure 3C), suggesting that carbohydrates are involved. This observation and the finding that both gp120 (Geijtenbeek et al., 2000) and ICAM-3-binding of DC can be inhibited by the anti-DC-SIGN antibodies AZN-D1 and -D2 suggest that ICAM-3 and gp120 bind to similar or closely located sites.

DC constitutes a heterogeneous population of cells, which are present at minute numbers in various tissues. To date, several subtypes of DC have been identified,

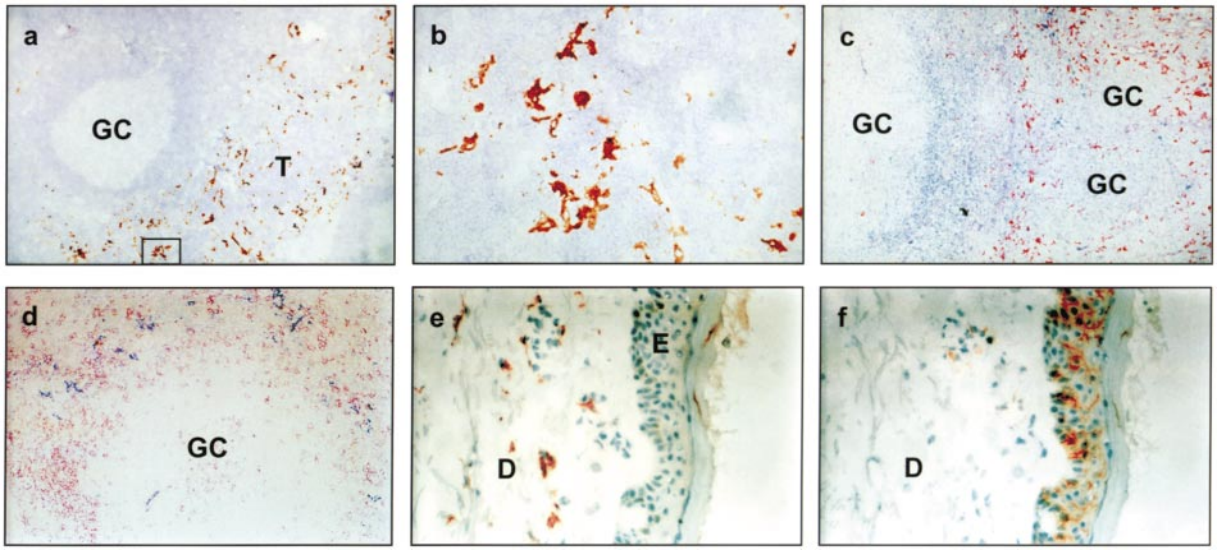


Figure 5. DC-SIGN Expression In Vivo Is Restricted to DC

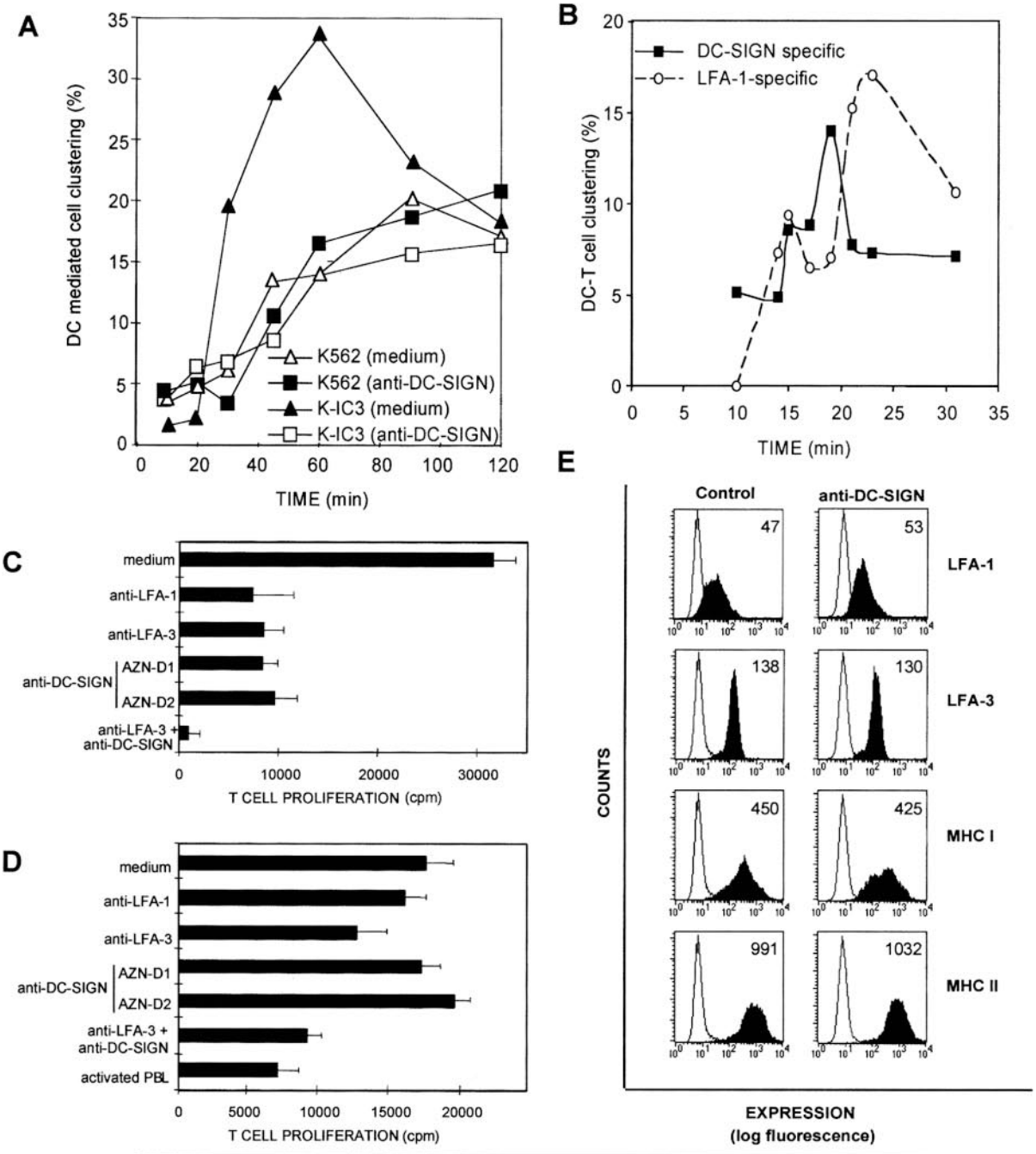
Immunohistochemical analysis of DC-SIGN expression in lymphoid tissue and human skin. Different tissue sections were stained with anti-DC-SIGN mAb: lymph node (a) and magnification of lymph node (b); tonsil (c); spleen (d; double labeling, CD3 [red] and DC-SIGN [blue]); skin (e); and Langerhans cells are identified by CD1a antibodies (f). The germinal center (GC), T cell area (T), epidermis (E), and dermis (D) are depicted. DC-SIGN positive cells are identified in the T cell areas of lymphoid tissues (a-d) and in the dermis of skin (e). Staining of serial sections demonstrates that these DC-SIGN positive cells do not express CD3, CD20, CD14, and CD68 (data not shown).

including myeloid DC derived from monocytes or $CD34^+$ bone marrow cells. To better define the different populations, much effort has been spent to generate antibodies that recognize DC lineage-specific cell surface molecules. So far, only a few antibodies are DC specific. These include DC-CK-1, DC-LAMP, CMRF-44, CMRF-56, and CD83 (Hock et al., 1994; Zhou and Tedder, 1995; Adema et al., 1997; de Saint-Vis et al., 1998). DC-SIGN can be added to this list since both immature and mature monocyte- and a subpopulation of $CD34^+$ bone marrow–derived DC express DC-SIGN (Table 1; data not shown). In situ DC-SIGN is exclusively expressed by DC subsets present in the T cell area of tonsils, lymph nodes, and spleen (Figure 5). Thus, DC-SIGN expression in situ correlates well with its function as an important mediator of DC–T cell clustering and T cell activation. In skin, DC-SIGN expression is found on dermal DC whereas it is not expressed by $CD1a^+$ Langerhans cells in the epidermis. In mucosal tissues such as rectum, uterus, and cervix, sites prone to HIV-1 exposure, DC-SIGN is abundantly expressed by DC present in the lamina propria, further substantiating the importance of the localization of DC as a first-line defense against viruses and pathogens (Geijtenbeek et al., 2000).

Interactions of DC with Resting T Cells

Recognition of antigen presented by professional antigen-presenting cells (DC) to T cells requires the formation of a specialized junction between these cells that is generated by carefully orchestrated recruitment of specific receptors into the contact area (Monks et al., 1998; Grakoui et al., 1999). It has been proposed that the first step requires low-avidity interactions of adhesion molecules such as LFA-1 and CD2 present on T cells to provide positional stability, thus facilitating TCR engagement (Shaw et al., 1986). TCR triggering subsequently initiates strong adhesive LFA-1-mediated interactions, through avidity alterations (Dustin and Springer, 1989; van Kooyk et al., 1989). Recently, Dustin et al. (1998) demonstrated that TCR signaling also leads to clustering of CD2 on T cells and enhanced LFA-3 binding, thereby reinforcing the contact between DC and T cells. Both the TCR and CD2 are, because of their smaller dimensions, localized in the center of the contact whereas the larger LFA-1 and ICAM-1 molecules form a ring around this central disc (Dustin et al., 1998; Grakoui et al., 1999). However, in most of these elegant studies interactions of resting T cells with the natural professional antigen-presenting cell, the DC, were not investigated. We propose a model in which the initial interaction of DC with resting T cells is mediated by DC-SIGN-ICAM-3 (Figure 6F), followed by interactions through other adhesion molecules such as LFA-1 and LFA-3 that are involved in more stable DC–T cell contacts (Figures 6A, 6B, and 6C). We hypothesize that the initial contact between DC and resting T cells mediated by DC-SIGN/ICAM-3 transiently stabilizes the intimate DC–T cell membrane contact to provide efficient TCR engagement (Figure 6F). The transient nature of DC-SIGN-ICAM-3 interactions enables DC to interact with a large number of resting T cells, until productive TCR engagement is obtained. TCR signaling alters the avidity of LFA-1 and CD2, thereby strengthening the interaction between DC and T cell via multiple adhesive contacts through LFA-1 and LFA-3 that will provide further positional stability and full activation of the T cell by the DC (Shaw and Dustin, 1997).

The importance of DC-SIGN/ICAM-3 interactions in the initial DC–T cell contact is further emphasized by


A
DC mediated cell clustering (%)
TIME (min)
K562 (medium)
K562 (anti-DC-SIGN)
K-IC3 (medium)
K-IC3 (anti-DC-SIGN)
B
DC-T cell clustering (%)
TIME (min)
DC-SIGN specific
LFA-1-specific
C
medium
anti-LFA-1
anti-LFA-3
anti-DC-SIGN
AZN-D1
AZN-D2
anti-LFA-3 + anti-DC-SIGN
T CELL PROLIFERATION (cpm)
D
medium
anti-LFA-1
anti-LFA-3
anti-DC-SIGN
AZN-D1
AZN-D2
anti-LFA-3 + anti-DC-SIGN
activated PBL
T CELL PROLIFERATION (cpm)
E
Control
anti-DC-SIGN
47
53
LFA-1
138
130
LFA-3
450
425
MHC I
991
1032
MHC II
COUNTS
EXPRESSION
(log fluorescence)
F
Dendritic cell - resting T cell interaction
T cell
DC
1 DC-SIGN
ICAM-3
2 ICAM-1
LFA-1
low avidity
3 MHC
TCR
LFA-3
CD2
4
high avidity

the potency of anti-DC-SIGN antibodies to inhibit DC-induced proliferation of resting T cells but much less of PHA-IL-2 activated T cells. Since, in contrast to resting T cells, activated T cells also express ICAM-1 and high CD2 levels (Alberola Ila et al., 1991; Parra et al., 1993), LFA-1/ICAM-1 and LFA-3/CD2 interactions take over DC-SIGN/ICAM-3 interactions. This observation stresses the importance of DC-SIGN in primary immune responses rather than secondary responses and directly correlates with its restricted tissue expression.

In summary, we have identified a novel DC-specific C-type lectin, DC-SIGN, that by binding to ICAM-3 plays an important role in establishing the first contact between DC and resting T cells. This interaction and subsequent low-avidity interactions of other adhesion molecules enable productive TCR engagement followed by adhesion strengthening. Future experiments are needed to determine how DC-SIGN-ICAM-3 interactions orchestrate the recruitment of adhesion molecules such as LFA-1 and CD2 and TCR molecules that are involved in the junction between antigen-presenting cells and resting T cells.

Experimental Procedures

Antibodies

The following mAb were used: NKI-L15 (anti-αL), AZN-L19 (anti-β2), AIIB2 (anti-β1), CBR-IC3/1, CBR-IC3/2, 186–269 (anti-ICAM-3), RR1/1 (anti-ICAM-1), CD14 (WT14), MHC class I (W6/32), and MHC class II (Q5/13) LFA-3 (TS2/9). Anti-DC-SIGN mAb AZN-D1 and AZN-D2 were obtained by screening hybridoma supernatants of human DC-immunized BALB/c mice for the ability to block adhesion of DC to ICAM-3, as measured by the fluorescent bead adhesion assay.

Cells

Immature DC were cultured from monocytes in the presence of IL-4 and GM-CSF (500 and 800 U/ml, respectively; Schering-Plough, Brussels, Belgium) (Romani et al., 1994; Sallusto and Lanzavecchia, 1994). At day 7, the phenotype of the cultured DC was confirmed by flow cytometric analysis. The DC express high levels of MHC class I and II, αMβ2 (CD11b), αXβ2 (CD11c), and ICAM-1, moderate levels of LFA-1 and CD86, and low levels of CD14. Peripheral blood lymphocytes (PBL) were obtained from buffy coats of healthy individuals and were purified using Ficoll density centrifugation. Transfection of COS7 was performed by DEAE dextran. Stable K562 transfectants expressing ICAM-3 (K562-ICAM-3) were generated by cotransfection of K562 with 10 μg PCRII ICAM-3 plasmid (gift from Dr. D. Simmons) and 2 μg PGK-hyg vector (te Riele et al., 1990) by electroporation as described (Lub et al., 1997).

Isolation and Expression of the cDNA Encoding DC-SIGN

Immunoprecipitated DC-SIGN was identified by peptide sequencing. Tryptic digestion, purification of the resulting peptides, and sequence analysis were performed by Eurosequence BV (Groningen, The Netherlands). The cDNA encoding the placenta gp120 binding C-type lectin was amplified by RT-PCR on total RNA from DC. PCR primers were based on the nucleotide sequence of the placenta gp120 binding C-type lectin (accession number M98457 [Curtis et al., 1992]), and the nucleotide sequences (5′ to 3′) are as follows: XF29, AGAGTGGGGTGACATGAGTG; and XR1265, GAAGT TCTGCTACGCAGGAG. The nucleotide sequence of the cloned cDNA was identical to that of placenta gp120 binding C-type lectin (Curtis et al., 1992). The cDNA was subsequently cloned into the eukaryotic expression vector pRc/CMV (pRc/CMV-DC-SIGN). Stable THP-1 transfectants expressing DC-SIGN were generated by transfection of THP-1 cells with pRc/CMV-DC-SIGN by electroporation similarly as was described for the generation of K562-ICAM-3.

Fluorescent Bead Adhesion Assay

Carboxylate-modified TransFluorSpheres (488/645 nm, 1.0 μm; Molecular Probes, Eugene, OR) were coated with ICAM-3-Fc as described previously (Geijtenbeek et al., 1999). The fluorescent bead adhesion assay was performed as described by Geijtenbeek et al. (1999). In brief, cells were resuspended in adhesion buffer (20 mM Tris-HCl [pH 8.0], 150 mM NaCl, 1 mM $CaCl_2$, 2 mM $MgCl_2$, and 0.5% BSA) at a final concentration of 5×10^6 cells/ml. Fifty thousand cells were preincubated with mAb (20 μg/ml) for 10 min at room temperature. Ligand-coated fluorescent beads (20 beads/cell) were

Figure 6. Transient DC-SIGN-Mediated Adhesion to ICAM-3-Expressing T Cells Is Essential in Allogeneic DC-Induced T Cell Proliferation

(A) Heterotypic cell clustering of DC with K562-ICAM-3 cells. K562 and K562 cells stable transfected with the cDNA encoding ICAM-3 (K562-ICAM-3) were labeled with the fluorescent dye hydroethidine. DC were labeled with the fluorescent dye sulfofluorescein. K562 and K562-ICAM-3 were incubated with DC (50×10^3 cells/cell type) with or without blocking anti-DC-SIGN antibody (AZN-D1; 20 μg/ml) at 37°C. At different time points, the heterotypic cell clustering was measured by flow cytometry. A representative experiment of two experiments is given.

(B) Dynamic cell clustering of DC with resting T cells is mediated by DC-SIGN. DC (50×10^3 cells) were preincubated with/without the anti-DC-SIGN antibodies (AZN-D1 and AZN-D2 [10 μg/ml] or anti-LFA-1 and anti-LFA-3 antibodies [10 μg/ml]) for 10 min at room temperature. Allogeneic T cells (1×10^6 cells), labeled with the fluorescent dye Calcein-A, were added, and the cell mixture was incubated at 37°C. The clustering was determined by flow cytometry by measuring the percentage of DC that clustered with fluorescently labeled T cells. A representative experiment out of three is shown.

(C) DC-SIGN-ICAM-3 interactions are important in DC-induced allogeneic T cell proliferation. Allogeneic resting responder T lymphocytes (100×10^3) were added to immature DC (1.5×10^3) with/without blocking mAb (10 μg/ml) against β2 integrins (AZN-L19), ICAM-1 (RR1/1), ICAM-3 (186–269), LFA-3 (TS2/9), and DC-SIGN (AZN-D1, AZN-D2). The cells were cultured for 4 days and pulsed for 16 hr with [^{3}H]methyl-thymidine, and incorporation was determined. Syngeneic DC-induced T cell proliferation is low (<5000 cpm). The results are expressed as the mean percent of CPM from triplicate wells. One representative experiment out of three is shown.

(D) DC-SIGN does not mediate interactions between DC and activated T cells. IL-2/PHA stimulated allogeneic responder T lymphocytes (100×10^3) were added to immature DC (1.5×10^3) with/without blocking mAb as described in Figure 6C. The cells were cultured for 4 days and pulsed for 16 hr with [3H]methyl-thymidine, and incorporation was determined. The results are expressed as the mean percent of CPM from triplicate wells. One representative experiment out of three is shown.

(E) Anti-DC-SIGN antibodies do not affect the DC phenotype. Immature DC were cultured for 1 day in the presence of anti-DC-SIGN antibodies or control antibodies (10 mg/ml). Cells were analyzed for expression of anti-LFA-1, -LFA-3, -MHC class I, and -MHC class II IgG2A antibodies, following staining with a FITC-conjugated goat anti-mouse IgG2A antibody. Cells were gated on forward and side scatter, and the mean fluorescence is shown in the top right corner of the histograms. One representative experiment out of three is shown.

(F) Model of DC-resting T cell interactions (1). DC-SIGN/ICAM-3 interactions form a first contact between DC and T cell. This transient interaction facilitates the formation of low-avidity interactions in which LFA-1/ICAM-1 also participates giving rise to loose antigen-independent DC–T cell clustering (2). Only after TCR ligation (3), signals result in high-avidity LFA-1/ICAM-1 and CD2/LFA-3 interactions giving rise to a further stabilization of the immunological synapse in which the smaller TCR/MHC/peptide and CD2/LFA-3 form the center and LFA-1/ICAM-1 form the outer ring (4).

added, and the suspension was incubated for 30 min at 37°C. Adhesion was determined by measuring the percentage of cells, which have bound fluorescent beads, by flow cytometry using the FACScan (Becton Dickinson, Oxnard, CA).

Heterotypic Cell Clustering and DC-Induced T Cell Proliferation

DC and ICAM-3-expressing cells (2×10^6 cells/ml) were labeled, respectively, with sulfofluorescein (Molecular Probes; 50 μg/ml) and hydroethidine (Molecular Probes; 40 μg/ml) for 1 hr at 37°C. DC and the ICAM-3-expressing cells were mixed (50×10^3 cells each) and incubated at 37°C. At different time points, the cells were fixed with paraformaldehyde (0.5%), and heterotypic cell clustering was measured by flow cytometry.

Clustering between DC with resting T cells was assessed by a different method. DC (50×10^3 cells) were preincubated with/without the anti-DC-SIGN mAb AZN-D1 and AZN-D2 (10 μg/ml) for 10 min at room temperature. Allogeneic PBL (1×10^6 cells), labeled with the fluorescent dye Calcein-A (Molecular Probes); 25 μg/10^7cells/ml for 30 min at 37°C were added and the cell mixture was incubated at room temperature. The clustering was determined by measuring percentage of DC that have bound fluorescent T cells by flow cytometry. The livegate was set to contain only DC.

Resting or activated (cultured in the presence of 400 U IL-2/ml 0.2 mg/ml PHA for 2 days) allogeneic responder T lymphocytes (100×10^3) were added to DC (1.5×10^3) in the presence of blocking mAb (10 μg/ml). The cells were cultured for 4 days. On day 4, the cells were pulsed for 16 hr with [3H]methyl-thymidine (1.52 TBq/mmol, 0.5 μCi/well; Amersham), and thymidine incorporation was quantified.

Immunohistochemical Analysis

Cryosections (8 μ) of the tissues were fixed in 100% aceton (10 min), washed with PBS, and incubated with the first antibody (10 μg/ml) for 60 min at 37°C. After washing, the final staining was performed with the ABC-PO/ABC-AP Vectastain kit (Vector Laboratories, Burlingame, CA) according to the manufacturer's protocol. Nuclear staining was performed with hematoxylin.

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