Retagging Identifies Dendritic Cell-specific Intercellular Adhesion Molecule-3 (ICAM3)-grabbing Non-integrin (DC-SIGN) Protein as a Novel Receptor for a Major Allergen from House Dust Mite*

Mohamed Emara†1, Pierre-Joseph Royer‡2, Jafar Mahdavi‡, Farouk Shakib‡3, and Amir M. Ghaemmaghami‡3

From the †School of Molecular Medical Sciences, Queen’s Medical Centre, The University of Nottingham, Nottingham NG7 2UH, United Kingdom and the ‡Respiratory Biomedical Research Unit, The University of Nottingham, Nottingham NG7 2UH, United Kingdom

Background: Allergen uptake by DCs is central to allergic sensitization.

Results: DC-SIGN recognizes major allergens from house dust mite and dog. However, silencing DC-SIGN leads to Th2 differentiation.

Conclusion: DC-SIGN is a newly identified receptor for Der p 1 and Can f 1 that appears to support Th1 cell differentiation.

Significance: Understanding of how allergic responses are selected and propagated is essential for developing novel therapies.

Dendritic cells (DCs) have been shown to play a key role in the initiation and maintenance of immune responses to microbial pathogens as well as to allergens, but the exact mechanisms of their involvement in allergic responses and Th2 cell differentiation have remained elusive. Using retagging, we identified DC-SIGN as a novel receptor involved in the initial recognition and uptake of the major house dust mite and dog allergens Der p 1 and Can f 1, respectively. To confirm this, we used gene silencing to specifically inhibit DC-SIGN expression by DCs followed by allergen uptake studies. Binding and uptake of Der p 1 and Can f 1 allergens was assessed by ELISA and flow cytometry. Intriguingly, our data showed that silencing DC-SIGN on DCs promotes a Th2 phenotype in DC/T cell co-cultures. These findings should lead to better understanding of the molecular basis of allergen-induced Th2 cell polarization and in doing so paves the way for the rational design of novel intervention strategies by targeting allergen receptors on innate immune cells or their carbohydrate counterstructures on allergens.

Dendritic cells (DCs) act as sentinels of the immune system and serve as a bridge between innate and adaptive immunity. Internalization of antigens by DCs is an important step in the sequence of events that leads to the induction of the adaptive immune response (1). DCs can efficiently sample their microenvironment using a plethora of receptors such as C-type lectin receptors (CLRs), Toll-like receptors, or scavenger receptors (2). Immature DCs take up antigens in peripheral tissues, process them into peptides, and then migrate to lymph nodes where they acquire a fully mature status capable of stimulating naïve T cells (3, 4). Immature DCs are characterized by their superior capacity for antigen uptake which can be attributed to the numerous CLRs that are highly expressed on these cells. These CLRs include mannose receptor (MR, CD206), dendritic cell-specific intracellular adhesion molecule (ICAM)-3-grabbing non-integrin (DC-SIGN, CD209), and dendritic and epithelial cell, 205 kDa (DEC-205, CD205) (5–7). C-type lectins are calcium-dependent carbohydrate-binding glycoproteins with a wide range of biological functions characterized by the presence of at least one carbohydrate recognition domain that interacts with and recognizes carbohydrates via either mannose or galactose side chains (8–11).

DCs have been shown to play a key role in the initiation and maintenance of immune responses to microbial pathogens as well as to allergens, but the exact mechanisms of their involvement in allergic responses and Th2 cell differentiation have remained elusive (12, 13). Given the importance of antigen recognition and uptake by DCs on downstream events leading to T cell differentiation, there is considerable interest in identifying potential receptors for allergens on DCs. Within this context, we and others have shown that MR is partially involved in the uptake of Der p 1, the major allergen from house dust mite (14, 15). Blocking MR by mannan (14) or its down-regulation using siRNA (15) leads to approximately 60–70% reduction in Der p 1 uptake by human monocyte-derived DCs. The residual uptake after blocking MR does not seem to be due to macrophagocytosis by DCs, and as such it is reasonable to suggest the presence of other putative allergen receptors on DCs. In this study, using a number of different approaches including confocal microscopy, receptor activity-directed affinity tagging (retagging) (16, 17) and gene silencing, we show that Der p 1 and Can f 1 uptake by human DCs is also mediated by DC-SIGN. Although down-regulation of MR inhibits Th2 differen-

Received for publication, October 12, 2011, and in revised form, December 14, 2011 Published, JBC Papers in Press, December 28, 2011, DOI 10.1074/jbc.M111.312520

© 2012 by The American Society for Biochemistry and Molecular Biology, Inc. Published in the U.S.A.
tiation (15, 18), intriguingly we have shown that knocking down DC-SIGN expression on human DCs leads to a bias toward Th2 cell differentiation in autologous DC-T cell co-cultures, suggesting an antagonistic relationship between the two main allergen receptors expressed on DC surface.

Early events at the interface of allergens and DCs play a key role in downstream events leading to allergic sensitization. Therefore, identifying receptors that are involved in the initial recognition and uptake of allergens by DCs would not only lead to better understanding of the molecular basis of allergen-induced Th2 cell polarization but also pave the way for the rational design of novel intervention strategies.

**EXPERIMENTAL PROCEDURES**

**Cell Preparation**—Immature DCs were generated from monocytes isolated from peripheral blood of nonatopic healthy donors (obtained with consent and after ethical committee approval) in the presence of interleukin (IL)-4 and granulocyte-macrophage colony-stimulating factor (GM-CSF) (250 units/ml and 50 ng/ml, respectively) (R&D Systems) in RPMI 1640 medium (Sigma-Aldrich) supplemented with 100 units/ml penicillin, 100 mg/ml streptomycin, 2 mM-l-glutamine (all from Sigma-Aldrich), and 10% low endotoxin FCS (AutoGen Bioclear, Calne, UK) for 6 days as described before (19). In some experiments, we used NIH-3T3 fibroblast transfectants stably expressing DC-SIGN (3T3/DC-SIGN) (a kind gift from Dr. Vineet KewalRamani, National Cancer Institute, Bethesda, MD) (20) and mock fibroblast cells lacking DC-SIGN.

**Confocal Microscopy**—To promote the adherence of DCs, glass base dishes (IWAKI, Asahi Techno Glass Corp., Japan) with an internal core diameter of 1 cm were coated with 6 µg/ml (w/v) of poly-L-lysine (Sigma) overnight. To deplete the cell membrane from cholesterol, immature DCs were treated with 10 mM (2-hydroxypropyl)-β-cyclodextrin (Sigma) which is a more water-soluble/toxicologically benign alternative to α-, β-, and γ-cyclodextrin (21–24). 5 × 10^7/200 µl β-cyclodextrin-treated or untreated DCs were then seeded in each dish for 1 h at 37 °C, 5% CO₂. Dishes were then placed under a Zeiss confocal microscope (LSM510uv META combi, Welwyn Garden City, UK) (equipped with a hot plate and tissue culture chamber) and received 5 µg/ml Cy5-labeled Der p 1 for 1. For each condition, confocal images were taken after 20 min.

**Retagging Technique**—Der p 1-binding proteins were purified as described previously for the Helicobacter pylori SabA adhesin (16, 17) with some modifications. Briefly, Der p 1, conjugated to the Sulfo-SBED multifunctional cross-linker (Pierce, Thermo Scientific), was incubated with DCs or monocytes according to the manufacturer’s recommendations. The photoactive cross-linker group was activated by 2 min of UV irradiation, and the biotin-(re)tagged proteins were purified with streptavidin-coated magnetic beads.

**Mass Spectrometry Analysis**—Extracted biotin-tagged proteins were separated by SDS-PAGE, and bands were digested with sequencing-grade trypsin (Promega) as described previously (19) and analyzed using a Micromass ToF-Spec E (Micromass). Briefly, the nanoflow LC-tandem MS was done on a 7-Tesla linear trap quadrupole-Fourier transform mass spectrometer (Thermo Electron) equipped with a nanospray source modified in-house. The spectrometer was operated in data-dependent mode, automatically switching to tandem MS mode. MS spectra were acquired in the FTICR, whereas tandem MS spectra were acquired in the linear trap quadrupole trap. For each scan of FTICR, the three most intense, doubly or triply charged, ions were sequentially fragmented in the linear trap by collision-induced dissociation. All the tandem mass spectra were searched using MASCOT PMF data base search engine (Matrix Science, London, UK) or Aldente (expasy site) against the databases (Sprot 54.0) for the human proteins as appropriate. Search parameters included a peptide mass accuracy tolerance of 0.2Da and allowed for modifications such as alkylation of cysteine during the tryptic digest procedure and the possible formation of methionine sulfoxide (19).

**Binding of Natural Allergens to Soluble DC-SIGN by ELISA**—ELISA-based binding assays were used to determine soluble DC-SIGN (sDC-SIGN; R&D Systems) binding to different allergens. All washing steps were performed in lectin buffer consisting of 10 mM Tris-HCl, pH 7.5, 10 mM CaCl₂, 0.154 M NaCl, and 0.05% (w/v) Tween 20. Maxisorp plates (Nunc, Thermo Scientific) were coated overnight with different test allergens or mannan (5 µg/ml) and blocked with 1% BSA. Plates were sequentially incubated with 5 µg/ml of sDC-SIGN and 5 µg/ml DC-SIGN mAb (clone 120507; R&D Systems). Bound antibodies were detected by incubation with phosphatase-conjugated goat anti-mouse Ab diluted to 1/1000, and p-nitrophenyl phosphate solution (Sigma) was added to the plates at concentration of 1 mg/ml. Absorbance was measured at 405 nm (Expert Plus, SLS, Nottingham, UK).

**Binding of Der p 1 to NIH-3T3 Cells Expressing DC-SIGN**—Allergens were labeled with Cy3 or Cy5 (GE Healthcare) labeling kits according to the manufacturer’s instructions and as described before (15). Binding assays were conducted in presence and absence of EDTA. 3T3/DC-SIGN fibroblasts were used to study binding of Der p 1 to DC-SIGN. Briefly, cells (2–10^9/100 µl) were incubated with Cy5 only or Cy5 Der p 1 at 37 °C for 45 min; cells were then washed and fixed in 0.5% formaldehyde. To investigate DC-SIGN specificity, NIH-3T3 fibroblasts that lack DC-SIGN expression (mock cells) were used, and binding was detected by flow cytometry on an Altra flow cytometer (Beckman Coulter).

**Blocking Experiments**—Immature DCs were resuspended in uptake medium (RPMI 1640 medium + 30% PBS with Ca²⁺ and Mg²⁺ + 10% FBS) and preincubated with (200 µg/ml) mannan (Sigma), or different concentrations (10 and 20 µg/ml) of Lewis-x (PAA-Lewis-x; Lectinity, Moscow, Russia) for 30 min at 37 °C and subsequently incubated with 5 µg/ml Cy5 Der p 1 for another 30 min. In some experiments, Cy5 Der p 1 was preincubated with varying concentrations (5, 10, and 20 µg/ml) of sDC-SIGN prior to addition to cells. BSA was used as a control. Cells were then washed and fixed in 0.5% formaldehyde, and Der p 1 uptake was determined by flow cytometry. Results are expressed as median fluorescence intensity (MFI).

**RNA Interference**—RNA interference (RNAi) was performed by transfecting DCs with small interfering RNA (siRNA) (50 nM), using the Lipofectamine RNAiMax (Invitrogen), to specifically knock down DC-SIGN. For DC-SIGN mediated gene silencing, the following oligonucleotides were employed:
DC-SIGN Recognizes Dust Mite Allergen

(GGCAUUGGCUGGACGACGACAAAU), and (AUUUGUCGUUCGGAGCCAUUGCC) (Invitrogen) and the nonsilencing control (CT) siRNA used was all star negative control (Qiagen). Gene knockdown was estimated quantitatively at message level by quantitative real time PCR (qRT-PCR) and at protein level by flow cytometry and Western blotting.

Real Time PCR—Real time PCRs were conducted using a MX3005P thermal cycler (Stratagene, Agilent Technologies), and PCR amplifications were performed with the SYBR Green method using the following primers: CD209 (forward, 5'-CCAAAGGAGGAGACAAGCAG-3' and reverse, 5'-GGAGCAGCAGCTCTGAGTTGA-3') and GAPDH (forward, 5'-GAGTGCAACGGATTTGGCTGT-3' and reverse, 5'-GACAAGCTTCCGGTTCTCAG-3'). Each reaction was performed in a final volume of 25 µl comprising 5 µl of template (cDNA), 12.5 µl of SYBR Green master mix (Stratagene), 1 µl (200 nm) of each primer, and 0.38 µl of ROX dye. Amplifications were performed starting with an initial denaturation step of 95 °C/30 s, followed by 40 cycles of 95 °C/30 s, 56 °C/60 s, and 72 °C/60 s.

Western Blotting—DC-SIGN knockdown was assayed by Western blotting as described elsewhere (25). Briefly, proteins were run on SDS-PAGE, transferred to nitrocellulose membranes (Amersham Biosciences, GE Healthcare), blocked with (PBS + 0.05% Tween 20 + 5% milk), and subsequently probed with rabbit polyclonal Ab to human CD209 (2 µg/ml; AbD Serotec, Kidlington, UK) or mouse anti-human mAb to β-actin (Sigma). Membranes were washed and reprobed with HRP-conjugated goat anti-rabbit IgG (Fc) or rabbit F(ab')2 anti-mouse IgG:HRP (AbD Serotec). Bands were visualized with ECL reagent (Amersham Biosciences), and the intensity of each protein was normalized against β-actin as an internal loading control. In retagging experiments the blots were probed with streptavidin directly conjugated with HRP (1:5000 dilution) (Thermo Fischer Scientific).

Effect of DC-SIGN Knockdown on Der p 1 and Can f 1 Uptake—DCs were transfected at day 1, and the effect of DC-SIGN gene silencing on uptake was studied. Briefly, DCs that have previously been treated with CT or DC-SIGN siRNA were washed in PBS, resuspended in uptake medium, and incubated with 5 µg/ml of either Cy5 Der p 1 or Cy3 Can f 1 for 30 min. Cells were then washed and fixed in 0.5% formaldehyde, and the quantitative uptake of Cy5 Der p 1 and Cy3 Can f 1 was then estimated by flow cytometry.

Effect of DC-SIGN Down-regulation on T Cell Polarization—Naïve (CD3+CD45RA+) T cells were isolated by immunomagnetic cell sorting (Miltenyi Biotec) according to the manufacturer’s instructions. DCs that had previously been treated with either CT or CD209 siRNA were loaded or not for 6 h with 5 µg/ml of either Cy5 Der p 1 or Cy3 Can f 1 for 30 min. Cells were then washed and fixed in 0.5% formaldehyde, and the quantity of uptake of Cy5 Der p 1 and Cy3 Can f 1 was then estimated by flow cytometry.

RESULTS

Glycoallergen Uptake by Human DCs Is a Receptor-mediated Event—Using cycloextran (27), we depleted the DC membrane from cholesterol thereby disrupting all receptor-mediated endocytosis (28). This led to almost complete abrogation of Der p 1 uptake by DCs compared with control cultures which were not treated by cycloextran (Fig. 1). We did not observe any changes in DC viability upon cycloextran treatment (data not shown). This observation together with our previous data (15) showing that MR is only responsible for 60–70% of Der p 1 uptake by human DCs clearly suggest the presence of other putative receptors on DCs that are capable of allergen uptake.

DC-SIGN Is Novel Receptor for Major House Dust Mite Allergen Der p 1—To identify other receptors on human DCs that are involved in Der p 1 uptake, we used retagging, a powerful tool for identifying cell surface receptors (16, 17). Using this technique, we were able to identify DC-SIGN as a novel receptor for Der p 1 (Fig. 2). Subsequently, the functional importance and contribution of DC-SIGN to uptake of allergens were assessed using a number of complementary approaches. First, we investigated the binding of DC-SIGN to different allergens using ELISA. Der p 1 (OD values = 1.416 ± 0.078), Can f 1 (1.153 ± 0.043), and Ara h 1 (OD = 0.930 ± 0.003), which was previously identified as DC-SIGN ligands (29), were all found to bind to DC-SIGN, albeit with different strengths, whereas the cat allergen Fel d 1 did not show any binding (Fig. 3).

A more physiologically relevant approach to study the binding of Der p 1 to DC-SIGN involved NIH-3T3 fibroblast transfectants stably expressing DC-SIGN. This showed that Cy5-labeled Der p 1 binds to NIH-3T3 fibroblasts expressing DC-SIGN (Fig. 4) but not mock cells as detected by flow cytometry.
Incubation with EDTA completely abrogated the binding, indicating that binding is Ca\(^{2+}\)-dependent. Furthermore, based on the carbohydrate specificity of Lewis-x for DC-SIGN (30, 31), we sought to block DC-SIGN on DCs using Lewis-x antigen to investigate further the impact of DC-SIGN on Der p 1 uptake by DCs. This resulted in a reduction of Der p 1 uptake by DCs. Results obtained (Fig. 5A) demonstrate that Der p 1 uptake was inhibited by Lewis-x in a dose-dependent manner with blocking reaching a plateau at 20 μg/ml (p < 0.05, n = 3). Mannose was used as a control and inhibited Der p 1 uptake by 60% which was in line with previous observations (14, 15).

Preincubation of Der p 1 with Soluble DC-SIGN Reduces Der p 1 Uptake by DCs in Dose-dependent Manner—We also sought to study the interaction between Der p 1 and DC-SIGN by flow cytometry through preincubation of Der p 1 with different concentration of sDC-SIGN. This resulted in a dose-dependent and statistically significant decrease in Der p 1 uptake (p < 0.05, n = 3) further confirming the role of DC-SIGN in recognition of Der p 1 (Fig. 5B). Similar concentrations of BSA did not lead to any changes in Der p 1 uptake (data not shown).

DC-SIGN Down-regulation Reduces Der p 1 and Can f 1 Uptake by DCs—To evaluate further the role of DC-SIGN in the uptake of Der p 1 and Can f 1 by DCs, we investigated the impact of DC-SIGN down-regulation following siRNA experiments. Here, we describe an efficient siRNA strategy for the systematic knockdown of DC-SIGN on human cells, as a simple and highly reproducible method. The quantitative measurement of gene silencing at the mRNA and protein levels was achieved via qRT-PCR (Fig. 6A), flow cytometry (Fig. 6, B and C), and Western blotting (Fig. 6D), obtaining percentage inhibition of 70–80%. In line with our previous studies (15, 32), DC viability after siRNA treatment as well as the expression and localization of MR was not affected by silencing DC-SIGN expression (data not shown). Allergen uptake data obtained following specific DC-SIGN gene silencing demonstrated a significant reduction in Der p 1 (32%; p < 0.01, n = 9) and Can f 1 (33%; p < 0.01, n = 4) uptake by DCs (Fig. 7). No effect was observed in the uptake of the negative control, FITC-SO₄³⁻-galactose, a specific ligand that is taken up by MR, confirming...
that the reduction in uptake obtained following siRNA treatment was DC-SIGN-mediated.

**DC-SIGN Down-regulation Favors Th2 Cell Polarization in DC-T Cell Co-cultures**—DCs that have previously been treated with either CT or DC-SIGN siRNA were loaded or not for 6 h with 5 \( \mu \)g/ml natural Der p 1 and cultured with CD3/CD45RA/CD11001 autologous T cells for up to 16 days. After restimulation with autologous DCs, the percentages of IL-4 and IFN-\( \gamma \) producing T cells were determined by intracellular staining. Given that the cells were isolated from peripheral blood of a nonatopic donor, not surprisingly both in the presence and absence of Der p 1 stimulation of naïve T cells by DC-SIGN-expressing DCs (CT-DCs) there was a Th1 bias as evidenced by high levels of IFN-\( \gamma \) production (Fig. 8). This is in line with previous observations showing that in healthy donors, the presence of natural Der p 1 dramatically enhanced Th1 polarization (33, 34). By contrast, our data show that silencing of DC-SIGN expression dampened the Th1 polarization in the unloaded as well as in the Der p 1 loaded condition mainly through a significant increase in IL-4 secretion as well as slight decrease in IFN-\( \gamma \) production. Interestingly, with the DC-SIGN-deficient cells, loading with Der p 1 resulted in a further reduction of the Th1 skewing.

**DISCUSSION**

We have previously shown that the uptake of a number of glycoallergens such as Der p 1 is mediated through MR; however, MR seems to be responsible for only approximately 60% of this uptake (15). Previous work (14) suggested that the residual Der p 1 uptake does not seem to be mediated through macropinocytosis, which is in line with our own observations (data not shown). Almost all endocytic pathways are sensitive to cholesterol perturbation (28), and clathrin-dependent (35) and -independent pathways (36) are both inhibited by the removal of cholesterol. In this study, using cyclodextran (27), we depleted the DC membrane from cholesterol thereby disrupting all receptor-mediated endocytosis (28). This led to almost complete abrogation of Der p 1 uptake by DCs compared with control cultures which were not treated by cyclodextran. These data clearly suggest the presence of other putative receptors on DCs that are capable of allergen uptake.

To identify such receptors we used retagging, which is mainly based on labeling the protein of interest with a multifunctional cross-linker composed of a biotin tag disulfide bonded to a photoactive group. Thus, allowing the labeled allergen to interact with the cells and then subjecting the cells to UV irradiation will enable the photoactive group to form a covalent bond with...
structures in the immediate vicinity. The bound receptor could then be extracted and identified by mass spectroscopy (16, 17). Using this technique, we identified DC-SIGN as a novel receptor for the major house dust mite and dog allergens, Derp1 and Can f 1, respectively.

Having identified DC-SIGN as a potential receptor for Derp1 on DCs, we set out to investigate its role in Derp1 uptake by DCs. The functional importance and contribution of DC-SIGN to Derp1 uptake by human DCs were assessed using different, but complementary approaches. First, we investigated the binding of DC-SIGN to different allergens using ELISA. Derp1, Can f 1, and Ara h 1 were all found to bind to DC-SIGN in contrast to the cat allergen Fel d 1 which showed no binding. Another, perhaps more physiologically relevant, approach is to study the binding of Derp1 to DC-SIGN using 3T3/DC-SIGN cells (20, 37). This showed that Cy5 Der p 1 binds to 3T3 fibroblasts expressing DC-SIGN, but not to mock cells as detected by flow cytometry. Incubation with EDTA completely abrogated the binding, indicating that binding is Ca²⁺/H¹-dependent.

The above binding data prompted us to investigate whether DC-SIGN is involved in allergen uptake by human DCs. Based on the carbohydrate specificity of Lewis-x for DC-SIGN (30, 31), we therefore sought to target DC-SIGN on DCs using a natural ligand (Lewis-x antigen) to investigate further the impact of DC-SIGN engagement on Derp1 uptake by DCs. Results obtained demonstrated dose-dependent and significant inhibition of Derp1 uptake by Lewis-x with nearly 20% inhibition with highest concentration (20 mg/ml) of Lewis-x.

DC-SIGN has also been shown to mediate the uptake of various allergens such as the major peanut allergen (Ara h 1) (29), as well as the Bermuda grass pollen allergen (BG-60) and the major group 2 allergen from house dust mite (Der p 2) (38). As such, this and other works clearly underscore the potential of CLRs (in particular MR and DC-SIGN), which are highly expressed on the surface of antigen-presenting cells, to serve as common receptors for recognition of a wide range of glycosylated allergens. Intriguingly, we have also shown for the first time that down-regulation of DC-SIGN expression through siRNA leads to a bias toward Th2 polarization in autologous DC-T cell co-cultures. This is in contrast with our earlier work showing that down-regulation of MR, a major Der p 1 receptor expressed on DCs, leads to an opposite effect, i.e. bias toward Th1 polarization (15, 18). This could be partly explained by data showing that differentiation of naive T cells toward Th2 occurs upon co-stimulation through ICAM-1 and ICAM-2 instead of ICAM-3, which is thought to be the main DC-SIGN counterstructure on T cells (39). Within this context, it is interesting to note that we have shown previously (37) that Der p 1, a cysteine protease, in its enzymatically active form, can cleave DC-SIGN (its “Th1-promoting” receptor) but not MR (its “Th2-promoting” receptor), and this will further amplify its allergenicity. Although the exact mechanism of polarized Th2 cell differentiation in the absence of DC-SIGN is yet to be determined, these data clearly indicate that glycoallergen uptake by DCs and events leading to downstream Th2 polarization are complex...
REFERENCES

1. Banchereau, J., Briere, F., Caux, C., Davoust, J., Lebecque, S., Liu, Y. J., Pulendran, B., and Palucka, K. (2000) Immunobiology of dendritic cells. Annu. Rev. Immunol. 18, 767–811
2. Figdor, C. G., van Kooyk, Y., and Adema, G. J. (2002) C-type lectin receptors on dendritic cells and Langerhans cells. Nat. Rev. Immunol. 2, 77–84
3. Lambrecht, B. N. (2001) Allergen uptake and presentation by dendritic cells. Curr. Opin. Allergy Clin. Immunol. 1, 51–59
4. Lanzavecchia, A. (1996) Mechanisms of antigen uptake for presentation. Curr. Opin. Immunol. 8, 348–354
5. Geijtenbeek, T. B., van Vliet, S. J., Engering, A., ’t Hart, B. A., and van Kooyk, Y. (2004) Self- and nonself-recognition by C-type lectins on dendritic cells. Annu. Rev. Immunol. 22, 33–54
6. van Kooyk, Y. (2008) C-type lectins on dendritic cells: key modulators for the induction of immune responses. Biochem. Soc. Trans. 36, 1478–1481
7. van Vliet, S. J., García-Vallejo, J. J., and van Kooyk, Y. (2008) Dendritic cells and C-type lectin receptors: coupling innate to adaptive immune responses. Immunol. Cell Biol. 86, 580–587
8. Cambi, A., Koopman, M., and Figdor, C. G. (2005) How C-type lectins detect pathogens. Cell Microbiol. 7, 481–488
9. Drickamer, K. (1999) C-type lectin-like domains. Curr. Opin. Struct. Biol. 9, 585–590
10. Drickamer, K., and Fadden, A. J. (2002) Genomic analysis of C-type lectins. Biochem. Soc. Symp. 69, 59–72
11. McGreer, E. P., Miller, J. L., and Gordon, S. (2005) Ligand recognition by antigen-presenting cell C-type lectin receptors. Curr. Opin. Immunol. 17, 18–24
12. Broide, D. H., Finkelman, F., Bochner, B. S., and Rothenberg, M. E. (2011) Advances in mechanisms of asthma, allergy, and immunology in 2010. J. Allergy Clin. Immunol. 127, 689–695
13. Eisenbarth, S. C., Pigott, D. A., and Bottomly, K. (2003) The master regulators of allergic inflammation: dendritic cells in Th2 sensitization. Curr. Opin. Immunol. 15, 620–626
14. Deslée, G., Charbonnier, A. S., Hammad, H., Angyalosi, G., Tille-Leblond, I., Mantovani, A., Tonnell, A. B., and Pestel, J. (2002) Involvement of the mannose receptor in the uptake of Der p 1, a major mite allergen, by human dendritic cells. J. Allergy Clin. Immunol. 110, 763–770
15. Royer, P. J., Emara, M., Yang, C., Al-Ghoulah, A., Tighe, P., Jones, N., Sewell, H. F., Shakib, F., Martinez-Pomares, L., and Ghaemmaghami, A. M. (2010) The mannose receptor mediates the uptake of diverse native allergens by dendritic cells and determines allergen-induced T cell polarization through modulation of IDO activity. J. Immunol. 185, 1522–1531
16. Ilver, D., Arnaqvist, A., Ogren, J., Frick, I. M., Kersulyte, D., Incecek, E. T., Berg, D. E., Covacci, A., Engstrand, L., and Borén, T. (1998) Helicobacter pylori adhesin binding fucosylated histo-blood group antigens revealed by retagging. Science 279, 373–377
17. Mahdavi, J., Sundén, B., Hurtig, M., Olaf, F. O., Forsberg, L., Roche, N., Angstrom, J., Larsson, T., Teneberg, S., Karlsson, K. A., Altraia, S., Wadström, T., Kersulyte, D., Berg, D. E., Dubois, A., Petersson, C., Magnusson, K. E., Norberg, T., Lindh, F., Lundskog, B. B., Arnaqvist, A., Hammarström, L., and Borén, T. (2002) Helicobacter pylori SabA adhesin in persistent infection and chronic inflammation. Science 297, 573–578
18. Emara, M., Royer, P. J., Abbas, Z., Sewell, H. F., Mohamed, G. G., Singh, S., Peel, S., Fox, J., Shakib, F., Martinez-Pomares, L., and Ghaemmaghami, A. M. (2011) Recognition of the major cat allergen Fel d 1 through the cysteine-rich domain of the mannose receptor determines its allergenicity. J. Biol. Chem. 286, 13033–13040
19. Horlock, C., Shakib, F., Mahdavi, J., Jones, N. S., Sewell, H. F., and Ghaemmaghami, A. M. (2007) Analysis of proteomic profiles and functional properties of human peripheral blood myeloid dendritic cells, monocyte-derived dendritic cells, and the dendritic cell-like KG-1 cells reveals distinct characteristics. Genome Biol. 8, R30
20. Wu, L., Martin, T. D., Vazeux, R., Unutmaz, D., and KewalRamani, V. N. (2002) Functional evaluation of DC-SIGN monocolonal antibodies reveals DC-SIGN interactions with ICAM-3 do not promote human immunodeficiency virus type 1 transmission. J. Virol. 76, 5905–5914
21. Gould, S., and Scott, R. C. (2005) 2-Hydroxypropyl-β-cyclodextrin (HP-β-CD): a toxicology review. Food Chem. Toxicol. 43, 1451–1459
22. Pontes Soares, C., Portilho, D. M. D., da Silva Sampiao, L., Einicker-Lamas, M., Morales, M. M., Costa, M. L., and Dos Santos Mermelstein, C. (2010) Membrane cholesterol depletion by methyl-β-cyclodextrin enhances the expression of cardiac differentiation markers. Cells Tissues Organs 192, 187–199
23. Potocnik, S. J., Jenkins, N., Murphy, T. V., and Hill, M. A. (2007) Membrane cholesterol depletion with β-cyclodextrin impairs pressure-induced contraction and calcium signaling in isolated skeletal muscle arterioles. J. Vasc. Res. 44, 292–302
24. van Gestel, R. A., Helms, J. B., Brouwers, J. B., and Gadella, B. M. (2005) Effects of methyl-β-cyclodextrin-mediated cholesterol depletion in porcine sperm compared to somatic cells. Mol. Reprod. Dev. 72, 386–395
25. Geijtenbeek, T. B., Torensma, R., van Vliet, S. J., van Duijnghoven, G. C., Adema, G. I., van Kooyk, Y., and Figdor, C. G. (2000) Identification of DC-SIGN, a novel dendritic cell-specific ICAM-3 receptor that supports primary immune responses. Cell 100, 575–585
26. Ghaemmaghami, A. M., Gough, L., Sewell, H. F., and Shakib, F. (2002) The proteolytic activity of the major dust mite allergen Der p 1 conditions dendritic cells to produce less interleukin-12: allergen-induced Th2 bias determined at the dendritic cell level. Clin. Exp. Allergy 32, 1468–1475
27. Gold, S., Monaghan, P., Mertens, P., and Jackson, T. (2010) A clathrin-independent macropinocytosis-like entry mechanism used by bluetongue virus-1 during infection of BHK cells. PLoS One 5, e11360
28. Mayor, S., and Pagano, R. E. (2007) Pathways of clathrin-independent endocytosis. Nat. Rev. Mol. Cell Biol. 8, 603–612
29. Shreffler, W. G., Castro, R. R., Kucuk, Z. Y., Charlop-Powers, Z., Grishina, G., Yoo, S., Burks, A. W., and Sampson, H. A. (2006) The major glycoprotein allergen from Arachis hypogaea, Ara h 1, is a ligand of dendritic cell-specific ICAM-3-grabbing non-integrin and acts as a Th2 adjuvant in vitro. J. Immunol. 177, 3677–3685
30. Appelmelk, B. J., van Die, I., van Vliet, S. J., Vandenbroucke-Grauls, C. M., Adema, G. I., and Borén, T. (2002) Recognition of the major cat allergen Fel d 1 through the cysteine-rich domain of the mannose receptor determines its allergenicity. J. Biol. Chem. 286, 13033–13040
31. Feinberg, H., Mitchell, D. A., Drickamer, K., and Weis, W. I. (2001) Structural basis for selective recognition of oligosaccharides by DC-SIGN and DC-SIGNR. Science 294, 2163–2166
32. Coelho, V., Krysov, S., Ghaemmaghami, A. M., Emara, M., Potter, K. N., Johnson, P., Packham, G., Martinez-Pomares, L., and Stevenson, F. K.
33. De Wit, D., Amraoui, Z., Vincart, B., Michel, O., Michils, A., Van Overveldt, L., Willems, F., and Goldman, M. (2000) Helper T cell responses elicited by Der p 1-pulsed dendritic cells and recombinant IL-12 in atopic and healthy subjects. *J. Allergy Clin. Immunol.* **105**, 346–352

34. Hammad, H., Charbonnier, A. S., Duez, C., Jacquet, A., Stewart, G. A., Tonnell, A. B., and Pestel, J. (2001) Th2 polarization by Der p 1-pulsed monocyte-derived dendritic cells is due to the allergic status of the donors. *Blood* **98**, 1135–1141

35. Subtil, A., Gaidarov, I., Kobylarz, K., Lampson, M. A., Keen, J. H., and McGraw, T. E. (1999) Acute cholesterol depletion inhibits clathrin-coated pit budding. *Proc. Natl. Acad. Sci. U.S.A.* **96**, 6775–6780

36. Naslavsky, N., Weigert, R., and Donaldson, J. G. (2004) Characterization of a nonclathrin endocytic pathway: membrane cargo and lipid requirements. *Mol. Biol. Cell* **15**, 3542–3552

37. Furmonaviciene, R., Ghaemmaghami, A. M., Boyd, S. E., Jones, N. S., Bailey, K., Willis, A. C., Sewell, H. F., Mitchell, D. A., and Shakib, F. (2007) The protease allergen Der p 1 cleaves cell surface DC-SIGN and DC-SIGNR: experimental analysis of in silico substrate identification and implications in allergic responses. *Clin. Exp. Allergy* **37**, 231–242

38. Hsu, S. C., Chen, C. H., Tsai, S. H., Kawasaki, H., Hung, C. H., Chu, Y. T., Chang, H. W., Zhou, Y., Fu, J., Plunkett, B., Su, S. N., Vieths, S., Lee, R. T., Lee, Y. C., and Huang, S. K. (2010) Functional interaction of common allergens and a C-type lectin receptor, dendritic cell-specific ICAM3-grabbing non-integrin (DC-SIGN), on human dendritic cells. *J. Biol. Chem.* **285**, 7903–7910

39. Bleijs, D. A., de Waal-Malefyt, R., Figdor, C. G., and van Kooyk, Y. (1999) Co-stimulation 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**, 2248–2258