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DC-SIGN promotes allergen uptake and activation of dendritic cells in patients with atopic dermatitis

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ABSTRACT

Background: Atopic dermatitis (AD) is a common inflammatory skin disease, concomitant with allergic reactions to allergens. However, the exact mechanisms of allergen-induced immune responses in AD are not clear. The aim of this study is to explore the role of DC-SIGN in capturing and processing glycan-containing allergens and in the subsequent DC activation and T helper cell polarization in AD patients. **Methods:** DC-SIGN expression on DCs from AD patients was analysed by confocal microscopy and flow cytometry. DC-SIGN binding to common allergens was determined by ELISA. Activation of monocyte-derived dendritic cells (Mo-DCs) by allergens was analysed by evaluation of pro-inflammatory cytokines production, and their impact on T-cell responses was investigated by a DC-T cell coculture.

Results: DC-SIGN expression was higher on DCs in the lesional skin of AD patients compared with that of healthy controls and was correlated with disease severity. DC-SIGN could bind to many common allergens including house dust mite allergen (Der p2) and egg white allergen (Gal d2). Mo-DCs showed measurable expression of DC-SIGN and a concentration-dependent uptake of Der p2 and Gal d2, which was inhibited by mannan and anti-DC-SIGN Abs. Der p2 and Gal d2 induced the production of pro-inflammatory cytokines, including TNF- α and IL-6, by DCs from AD patients and facilitated Th2 and Th22 cell polarization.

Conclusions: Binding of common allergens by DC-SIGN on DCs may initiate allergen sensitization of AD or provoke the relapse of AD. Regulating the allergen-DC-SIGN interaction might be a promising strategy to prevent or intervene in the progress of AD.

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1. Introduction

Atopic dermatitis (AD) is a common inflammatory skin disease. Most AD patients have higher levels of specific IgE Abs against allergens that have been shown to initiate or provoke the relapse of AD. However, how the allergen is originally recognized by the immune system and how the immune response is induced is incompletely defined.

Abbreviations: AD, atopic dermatitis; CLRs, C type lectin receptors; DCs, dendritic cells; DC-SIGN, dendritic cell-specific ICAM-3-grabbing non-integrin; Mo-DCs, monocyte-derived dendritic cells; MFI, mean fluorescence intensity; IL, interleukin; TNF, tumour necrosis factor; TLRs, Toll like receptors; LPS, lipopolysaccharide; FACS, flow cytometry.

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As specialized antigen presenting cells, DCs have the primary role of recognizing and processing allergens to induce an adaptive immune response and participating in the pathogenesis of AD. DCs constantly move around and sense their environment through a variety of pattern-recognition receptors, such as C-type lectin receptors (CLRs), Toll-like receptors (TLRs) or scavenger receptors [1]. Among these receptors, CLRs are crucial in the recognition of complex glycan structures on various pathogens and allergens. Immature DCs use several highly expressed CLRs for antigen uptake, including the mannose receptor (MR) [2], DC receptor (DEC-205) [3,4], Langerin [5], and DC-specific intercellular adhesion molecule 3-grabbing nonintegrin (DC-SIGN), or through Fc receptors, such as Fc ϵ RI and Fc γ RI.

The interaction between allergens and CLRs has not been fully elucidated, but the fact that most allergens contain complex glycan structures raises the possibility that the allergen-CLR interaction may modulate the function of DCs and the subsequent immune response. Indeed, complex glycans are commonly present in plant,

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insect, and food allergens [6] and have contributed much of the cross-reactive IgE recognition [7–9]. DC-SIGN [10] contains a C-type carbohydrate-recognition domain and is known to bind to high mannose oligosaccharides and several fucosylated Lewis-like glycoforms with different binding patterns [11]. To investigate the role of the allergen-DC-SIGN axis in AD, we designed experimental strategies to prove the hypothesis that allergens interact with DC-SIGN and subsequently modulate DC functions and induce Th2 polarization in AD patients.

2. Materials and methods

2.1. Patient information

The study was approved by the ethics committee of the Institute of Dermatology in Peking Union Medical College. Twenty adult AD patients (aged 18–65 years, mean age 32 years) with AD fulfilling the criteria of Hanifin and Rajka were enrolled. The distribution of age between the patient group and the control group could change to comply with normal distribution if the standard test is transformed from 0.05 to 0.01, and there was no statistical difference between the two groups ($P > 0.01$). For the study on DC-SIGN expression in AD lesions, biopsies were acquired from five mild AD patients (mean SCORAD = 13) and five severe AD patients (mean SCORAD = 68). Normal skin tissue samples were obtained from age- and sex-matched healthy individuals undergoing plastic surgery. For the study of cytokine expression by Mo-DCs, 20 AD patients (mean SCORAD = 32) were included, with 10 patients who were allergic to house dust mites and 10 patients who were allergic to egg whites.

2.2. Isolation of epidermal and dermal cells

Skin tissue pieces were floated on a solution of 1.2 U/ml Dispase (Roche Applied Bioscience, Almere, Netherlands) in RPMI for 90 min at 37 °C to separate the dermis and epidermis. The epidermis was digested in 0.25% trypsin, and the dermis was digested in 500 U/ml collagenase D (Worthington Biochemicals, Lakewood, NJ) in RPMI for 45 min at 37 °C and then passed through a 70 μ m filter.

2.3. Immunofluorescence and confocal microscopy

Sections of frozen OCT-embedded skin tissues were blocked with 10% FBS and were incubated with mouse anti-human CD11c or rabbit anti-human DC-SIGN (Abcam); then the sections were incubated with Alexa Fluor 488-conjugated donkey anti-mouse or Alexa Fluor 647-conjugated donkey anti-rabbit IgG (Invitrogen). After staining with DAPI (Roche Diagnostics), the sections were imaged by confocal microscopy (Olympus BX-50).

2.4. Binding assays of allergen extract with soluble DC-SIGN

Microplates were coated with varying concentrations of allergen extracts (Beijing Macro-Union Pharmaceutical Co., Ltd.) and were blocked with 10% FBS. The plates were then incubated with 1 μ g/ml recombinant soluble human DC-SIGN-Fc (R&D Systems, Minneapolis, MN), followed by incubation with 3 μ g/ml HRP-conjugated goat anti-human IgG Fc Abs (Millipore). EDTA (10 mM) was added to test the blocking effect, and mannan and BSA were used as the positive and negative controls, respectively.

2.5. Binding of Der p2 or Gal d2 to HEK 293T cells expressing DC-SIGN

The vector pCMV-EGFP-DC-SIGN was constructed by inserting the DC-SIGN-encoding fragment into the pCMV-EGFP plasmid.

HEK 293T cells were transfected with the pCMV-EGFP-DC-SIGN plasmid, and expression of DC-SIGN and EGFP on the transfected HEK293T cells was analysed by flow cytometry (FACS) and confocal microscopy. Varying concentrations of Alexa Fluor 647-labeled Der p2 or Gal d2 (INDOOR Biotechnologies, Charlottesville, VA) were incubated with HEK 293T cells transfected with DC-SIGN (2×10^5 cells/100 μ l) at 37 °C for 15 min or 45 min. The binding assays were conducted in the presence or absence of EDTA and were analysed by a FACS VERSE flow cytometer (BD Biosciences).

2.6. Mo-DCs generation, antigen uptake and inhibition assays

CD14⁺ monocytes were separated from PBMC by a MACS separator (Miltenyi, autoMACSPro) using the monocyte isolation kit (MACS, Miltenyi Biotec) and were cultured with 200 ng/ml GM-CSF and 10 ng/ml IL-4 (R&D Systems) to obtain DCs as described before [12,13]. Collected Mo-DCs were incubated with Alexa Fluor 647-labeled Der p2 or Gal d2 (20 μ g/ml) for 15 min at 37 °C and analysed by FACS and a confocal laser scanning microscope system (FluoViewTM FV 1000, Olympus corporation). For anti-DC-SIGN-blocking experiments, Mo-DCs were pre-incubated with different concentrations of anti-DC-SIGN blocking antibody (R&D System) or mannan (sigma) for 10 min at 37 °C before incubation with Der p2 or Gal d2.

2.7. RNA interference

RNA interference (RNAi) was performed by transfecting Mo-DCs with DC-SIGN-specific small interfering RNA (50 nm) using Lipofectamine RNAiMax (Invitrogen). The following oligonucleotides were employed: sense: 5'-GGCAAUGGUGGACGACGACAAAU-3' and antisense: 5'-AUUUGUCGUCGUCCAGCCAUUGCC-3' (Invitrogen). Efficiency of gene knockdown was determined by quantitative real-time PCR (qPCR), FACS and Western blotting.

2.8. Activation of Mo-DCs by Der p2 or Gal d2

House dust mite allergen or egg white allergen induced Mo-DC activation was determined by evaluation of IL-1 β , IL-12p70, IL-6 and TNF- α secretion in the supernatant of cultured Mo-DCs when exposed to Der p2 or Gal d2 (20 μ g/ml). The levels of cytokine expression were measured by ELISA according to the manufacturer's instructions (eBioscience). Anti-DC-SIGN blocking antibody (5 μ g/ml) (R&D, MAB161) and/or MyD88 inhibitory peptide, Pepinh-MYD (5 μ g/ml) (Invivogen, Catalog # tlr1-pimy) were used for examining the involvement of DC-SIGN or TLRs in cytokine production.

2.9. DC-T cell coculture

After 48 h of stimulation with Der p2 or Gal d2, 3×10^5 Mo-DCs were cultured with 1.5×10^6 purified CD4⁺CD45RA⁺T cells purified by the naïve T cell selection kit (MACS) from PBMC of the same donor. On day 5, rh-IL-2 (20 μ /ml, R&D System) was added to the culture and was added every three days from then on. On day 14, the supernatant of the culture was harvested for IL-4/IFN- γ /IL-17A/IL-22 measurement.

2.10. Statistical analysis

Statistical analyses were performed with GraphPad Prism 4.0 (GraphPad Software, San Diego, CA), and data were analysed with the nonparametric test (Kruskal-Wallis). The number of independent experiments was equal to or greater than three. The results were expressed as the means $\pm$ S.E and were considered statistically significant when the P -value was < 0.05 .

3. Results

3.1. Elevated expression of DC-SIGN on dermal DCs from patients with AD

To examine DC-SIGN expression in AD lesions, the dermis and epidermis were carefully separated from the skin biopsies of AD patients and healthy controls, and single-cell suspension was produced and analysed for the expression of DC-SIGN by FACS. While CD11c⁺DC-SIGN⁺ cells were not observed in the epidermis of AD patients and healthy controls, the percentages of DC-SIGN⁺ cells among CD11c⁺ cells in the dermis were 47.1% and 1.7% in AD patients and healthy controls, respectively (Fig. 1A). The expression level of DC-SIGN on CD11c⁺ cells of AD patients was higher than that of healthy controls, as indicated by MFI (Fig. 1A). Severe AD had more CD11c⁺DC-SIGN⁺ cells and a higher surface number of DC-SIGN than that of mild AD. We also compared the number of CD11c⁺DC-SIGN⁺ cells and expression levels of DC-SIGN on DCs between AD patients and healthy controls by using confocal

microscopy. The data showed that the number of CD11c⁺DC-SIGN⁺ cells was much higher in AD patients than that of healthy controls, which was correlated with the disease severity ($p < 0.05$); DC-SIGN staining on dermal DCs in AD patients was brighter than that of healthy controls (Fig. 1B).

3.2. DC-SIGN is a receptor for a variety of allergens

First, we analysed the binding of DC-SIGN to a variety of allergens using ELISA, in which the allergens were used as coating antigens. The result showed that recombinant soluble DC-SIGN could bind to all of the allergens used with variable binding abilities (Fig. 2). Second, we used a more physiologically relevant approach to study the binding of DC-SIGN to Der p2 and Gal d2 by using HEK 293T transfectants expressing DC-SIGN-EGFP. The FACS result showed that Der p2 could bind to HEK 293T transfectants (Fig. 3A), and the immunofluorescence result showed that Alexa Fluor 647-labeled Der p2 co-localized with DC-SIGN-EGFP on HEK 293T transfectants (Fig. 3B), indicating that Der p2 binds to DC-

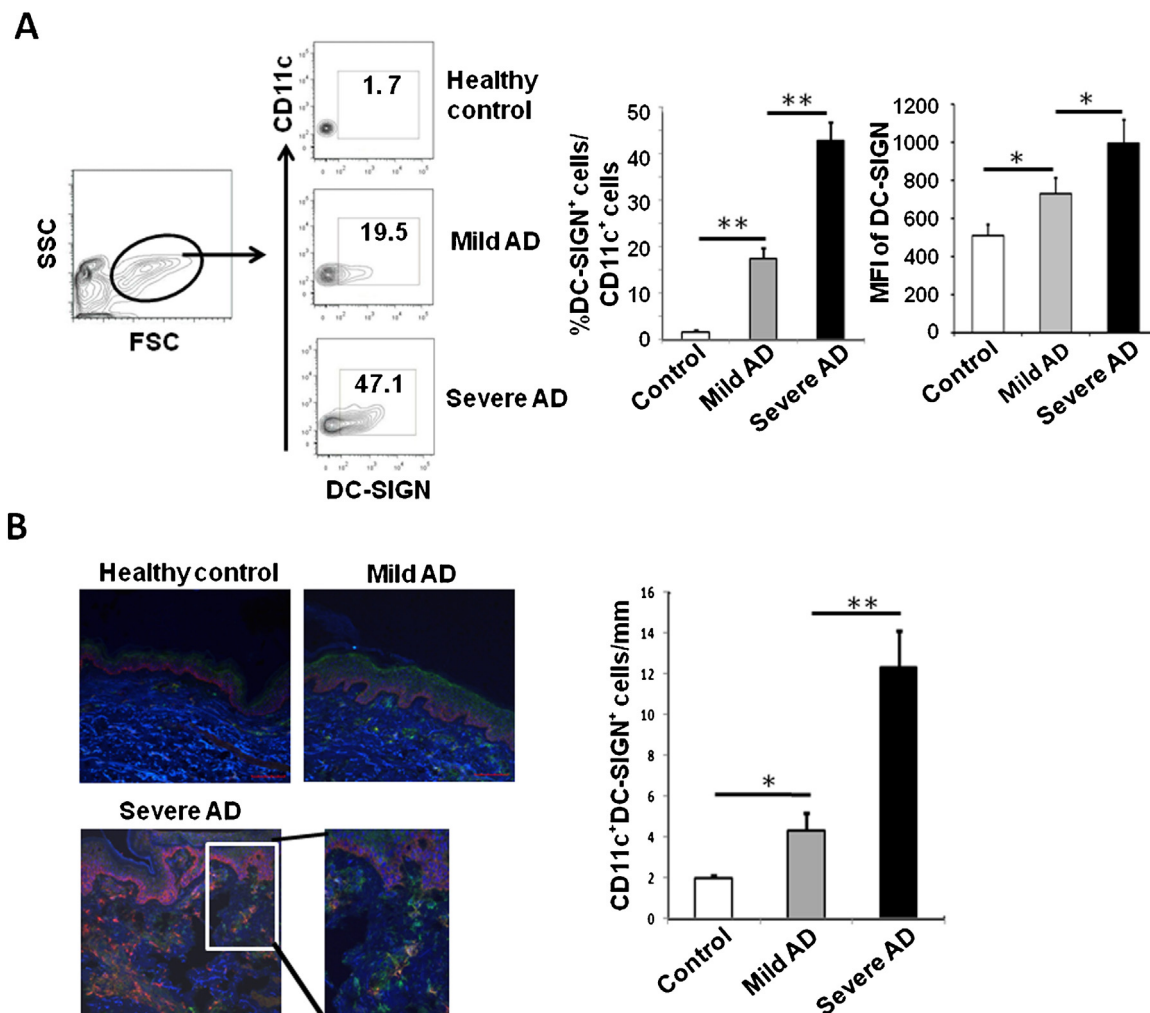


Fig. 1. Evaluation of CD11c⁺DC-SIGN⁺ dermal DCs from patients with atopic dermatitis. (a) FACS result showing the percentage of CD11c⁺ DC-SIGN⁺ cells in the dermis of AD patients and healthy controls. Data are representative of ten patients and five controls. Bar-graph displaying the statistical results of the percentage of DC-SIGN positive cells in the CD11c⁺ population in dermis. MFI of DC-SIGN on CD11c⁺ DC-SIGN⁺ cells is also shown in the bar-graph. The results are expressed as the means $\pm$ S.E. * $P < 0.05$, ** $P < 0.01$. (b) Immunofluorescence staining of CD11c⁺DC-SIGN⁺ dermal DCs cells in the skin biopsy of AD patients and healthy controls under high-power view (green: CD11c, red: DC-SIGN). Original magnification: 200 $\times$. Scale bar = 100 μ m. The bar-graph shows the number of DC-SIGN/CD11c double-positive cells per high-power field for five fields in the papillary dermis. The results are expressed as the means $\pm$ S.E. * $P < 0.05$, ** $P < 0.01$.

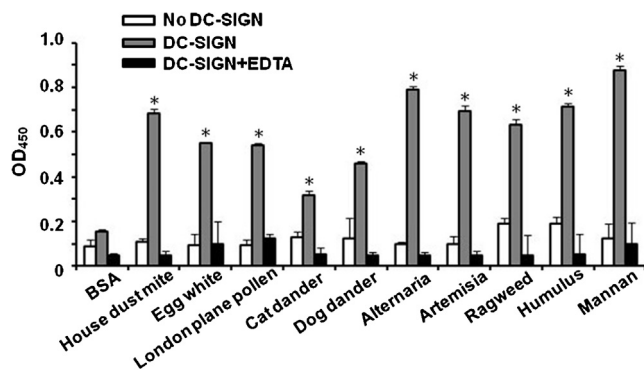


Fig. 2. Binding of natural allergens to soluble DC-SIGN-Fc revealed by ELISA. One hundred micrograms per millilitre of allergens, including house dust mite, egg white, cat dander, dog dander, Alternaria, London plane pollen, Artemisia pollen, ragweed pollen, and Humulus (Hop) extracts, were coated overnight, then probed with soluble DC-SIGN-Fc. Mannan and BSA were used as positive and negative controls, respectively. Inhibition assays were performed by incubation with 5 mM EDTA for 1 h prior to adding the allergens. The relative binding activity was expressed as optical density (OD). Data represent results from at least three independent experiments. The significant differences between groups were indicated with asterisks. * $P < 0.05$.

SIGN on the surface of HEK293T cells. We obtained a similar result when Alexa Fluor 647-labeled Gal d2 was used. When EDTA, a calcium chelator, was present during the incubation, neither Der p2 nor Gal d2 could bind DC-SIGN, indicating that binding is Ca^{2+} dependant.

3.3. DC-SIGN mediated Der p2 and Gal d2 uptake by DCs

Next, we explored the role of DC-SIGN in the uptake of allergens by DCs. Mo-DCs were incubated with Alexa Fluor-conjugated Der p2 or Gal d2 and subjected to FACS and immunofluorescence analyses. The MFI of Mo-DCs after incubation with Alexa Fluor-conjugated Der p2 or Gal d2 was much higher than that of healthy controls, as shown in Fig. 4A and B, indicating that there was substantial uptake of Der p2 and Gal d2 by Mo-DC. Because mannan is a non-activating ligand for DC-SIGN, we sought to block DC-SIGN on Mo-DCs using mannan. There was a dose dependant reduction in the uptake of Der p2 and Gal d2 by Mo-DCs as indicated by reduced MFI of Mo-DCs incubated with Alexa Fluor-conjugated Der p2 or Gal d2 in the presence of mannan ($p < 0.05$, $n = 5$), suggesting that DCs could uptake Der p2 and Gal d2 via DC-SIGN (Fig. 4A). Additionally, a reduction of Der p2 and Gal d2 uptake was observed when Mo-DCs were pre-incubated with DC-SIGN-blocking Abs or EDTA (Fig. 4B).

To confirm the role of DC-SIGN in the uptake of Der p2 and Gal d2 by DCs, we analysed the uptake of allergens by Mo-DCs with DC-SIGN knockdown. As shown in Fig. 4C and D, a 70% reduction of DC-SIGN expression at both the mRNA and protein levels was observed after DC-SIGN-specific siRNA was applied to Mo-DCs. Importantly, there was a significant reduction in MFI of Mo-DCs incubated with Der p2 (76%, $p < 0.05$, $n = 10$) and Gal d2 (77%, $p < 0.05$, $n = 10$) in the group of DC-SIGN silencing, indicating that uptake of Der p2 and Gal d2 by DCs were mediated by DC-SIGN (Fig. 4E).

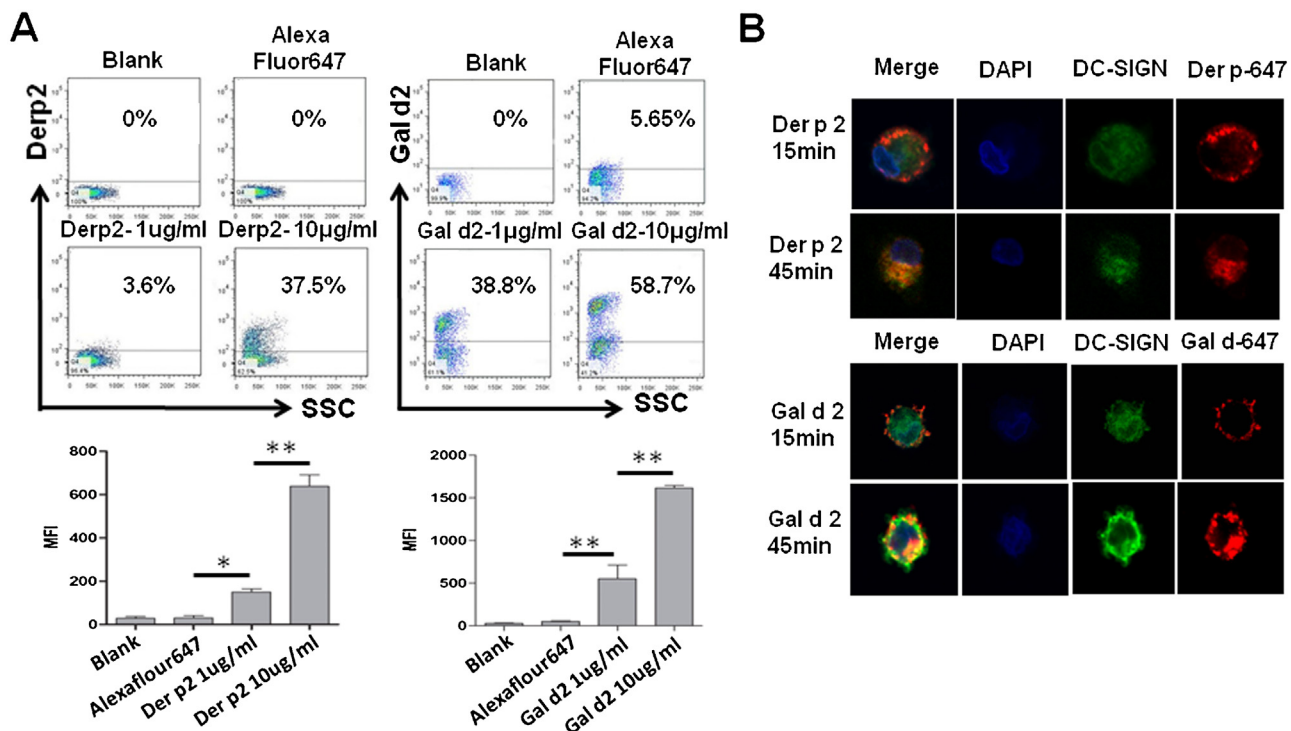


Fig. 3. Binding of Der p2 and Gal d2 to HEK293T cells expressing DC-SIGN-EGFP. (a) HEK 293T cells were transfected with DC-SIGN-EGFP-expressing plasmid and incubated with Alexa Fluor 647-conjugated Der p2, Gal d2 or Alexa Fluor 647 alone. FACS shows the binding and/or uptake of Der p2 or Gal d2 by DC-SIGN-expressing HEK 293T cells. Different doses of Der p2 and Gal d2 were used. Bar-graphs display the statistical results of MFI of DC-SIGN-expressing HEK 293T cells after incubation with Alexa Fluor 647-conjugated Der p2 or Gal d2. (b) HEK 293T cells were transfected with DC-SIGN-EGFP-expressing plasmid and were incubated with Alexa Fluor 647-conjugated Der p2, Gal d2 or Alexa Fluor 647 alone. The allergen binding and/or uptake by DC-SIGN-expressing HEK293T cells were determined by immunofluorescence at different time points. Green: DC-SIGN, red: Der p2 or Gal d2, blue: DAPI. Data are representative of results from at least three independent experiments.

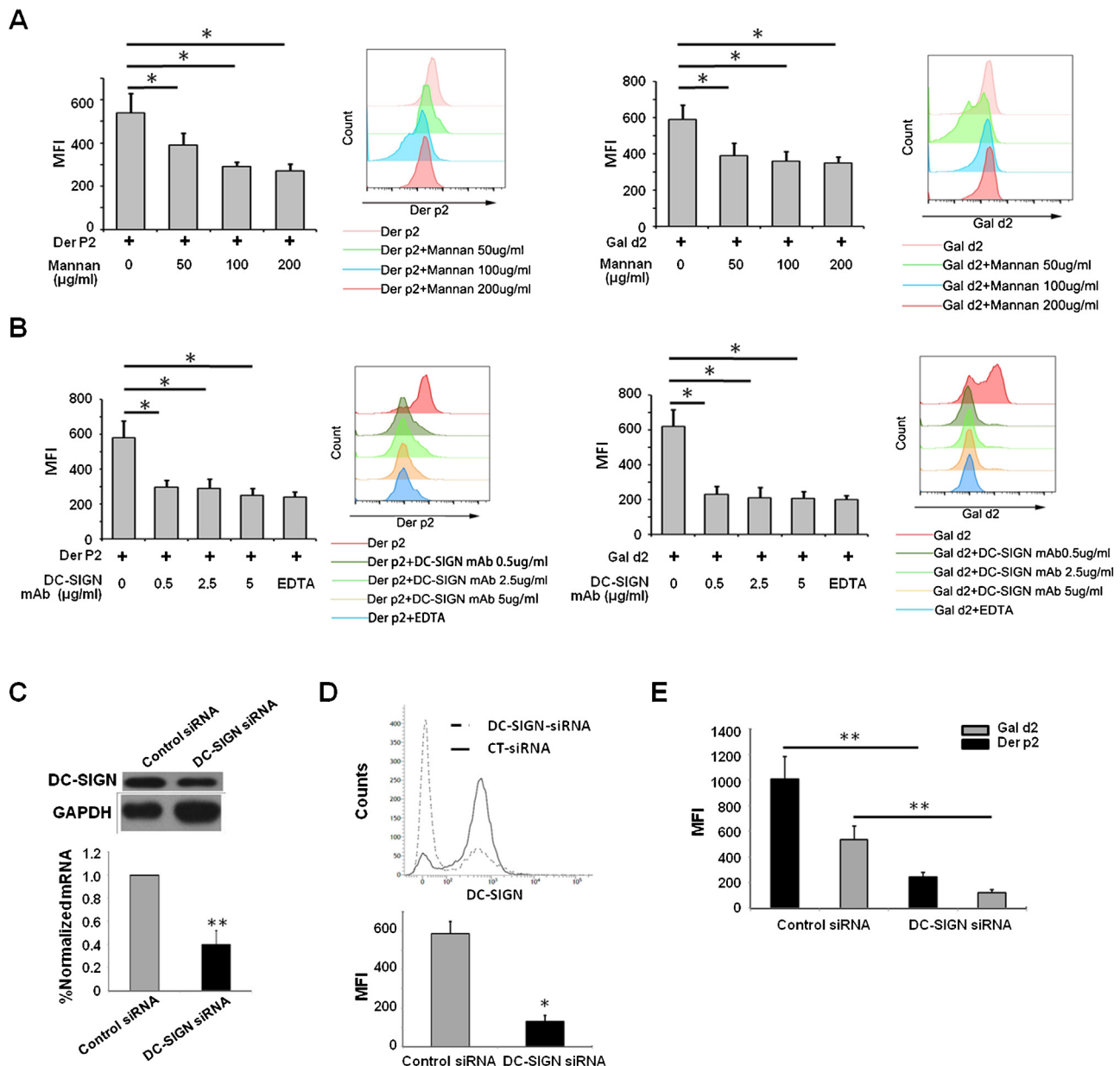


Fig. 4. Mannan and DC-SIGN blocking Abs reduced the uptake of Der p2 and Gal d2 by DCs. (a and b) Mo-DCs were incubated with Alexa Fluor-conjugated Der p2 or Gal d2 in the presence or absence of increasing concentrations of Mannan or DC-SIGN blocking Abs, respectively and were then subjected to FACS analysis. Inhibition assays were performed by incubation with 5 mM EDTA for 1 h prior to adding the allergens. Data shown are the histogram and MFI from the FACS result. (c and d) Mo-DCs were transfected with DC-SIGN-specific siRNA or control siRNA; DC-SIGN expression was quantitatively analysed by Western blot, qRT-PCR and FACS. (e) siRNA-transfected Mo-DCs were incubated with Alexa Fluor-conjugated Der p2 or Gal d2 for allergen uptake and were subjected to FACS analysis. Data are representative of results from at least three independent experiments. Statistical results of the MFI are shown as the means $\pm$ S.E. * $P < 0.05$, ** $P < 0.01$.

3.4. Der p2 and Gal d2 induced cytokine production by Mo-DCs via DC-SIGN

To examine the functional effects of Der p2 and Gal d2 on the activation of DCs in AD patients, Mo-DCs from a total of 20 patients with AD were analysed. The results showed that Der p2 or Gal d2 at 20 μ g/ml were able to significantly induce TNF- α , IL-6 and IL-1 β expression by Mo-DCs but not IL-12p70 expression by Mo-DCs (Fig. 5). Addition of anti-DC-SIGN blocking Abs at 5 μ g/ml was able to decrease Der p2- or Gal d2-induced TNF- α and IL-6 expression to 30–45% but did not decrease IL-1 β expression. Nonspecific effects of anti-DC-SIGN Abs were excluded by using isotype control Abs (Supplementary Fig. 1). Considering the potential involvement of TLR4 in an allergen-mediated effect on DCs, we used MyD88

inhibitory peptide to block the signalling pathway of TLRs and found a 45–55% reduction in the levels of TNF- α and IL-6 secretion after the addition of MyD88 inhibitory peptide.

3.5. Der p2 and Gal d2 activated DCs and primed Th2 and Th22 response in DC-SIGN dependant manner

Lastly, we tested the hypothesis that Der p2 and Gal d2 could activate DCs to preferentially prime naïve T cells towards a Th2 phenotype. Mo-DCs from AD patients and healthy controls stimulated with Der p2 or Gal d2 were cultured with naïve autologous T cells for two weeks. The T cell phenotype was analysed by cytokine measurement for IL-4, IL-22, IL-17A and IFN- γ . Consistent with results from previous reports, in the presence of

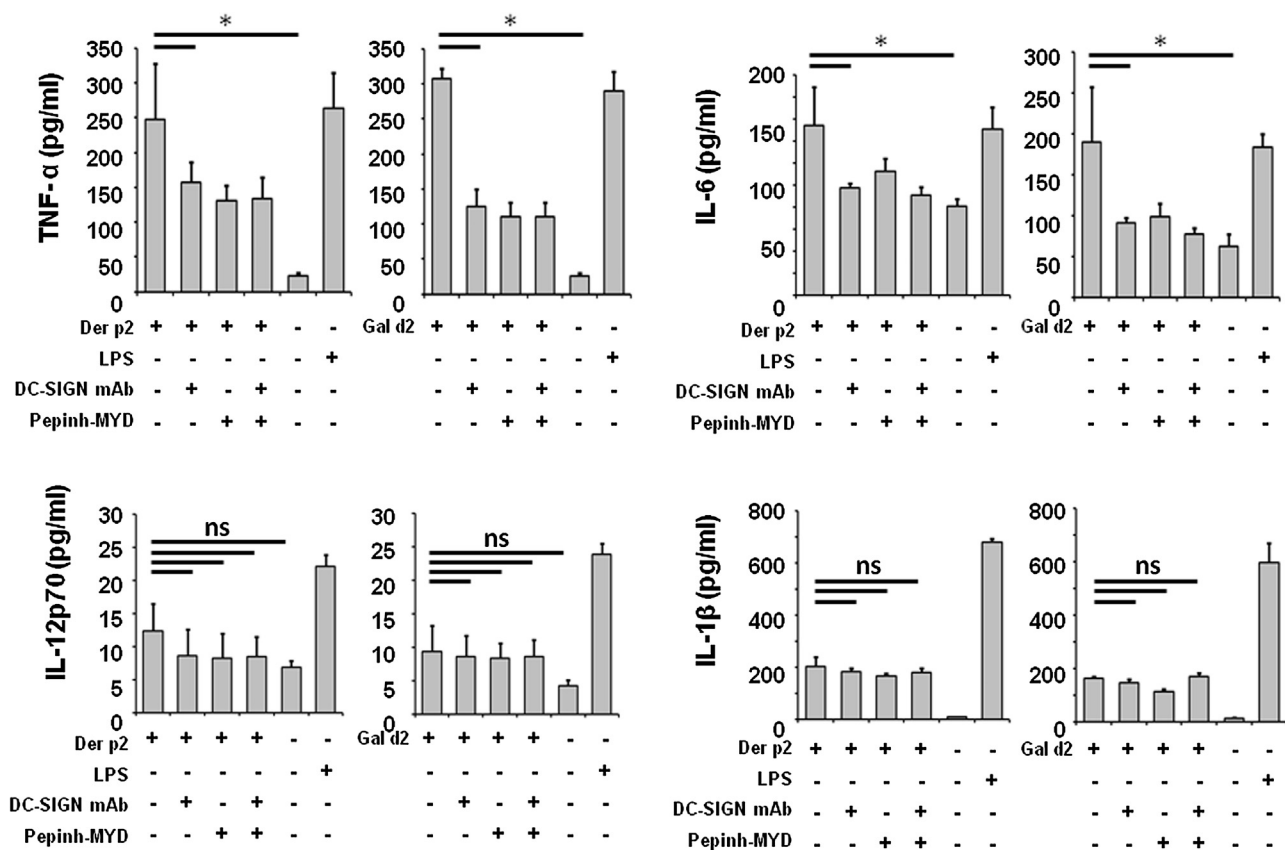


Fig. 5. Expression of TNF- α , IL-6, IL-12p70 and IL-1 β by Mo-DCs stimulated with Der p2 and Gal d2. Mo-DCs were incubated with Der p2 or Gal d2 for 24 h, and the supernatant was subjected to TNF- α (a), IL-6 (b), IL-12p70 (c) and IL-1 β (d) analysis by ELISA. DC-SIGN-specific blocking mAb was used to block the binding of Der p2 to DC-SIGN, and MyD88 inhibitory peptide was used to block the MyD88 signalling pathway. Data represent results from at least three independent experiments and are expressed as the means $\pm$ S.E. * P < 0.05, ** P < 0.01.

Der p2 or Gal d2-stimulated DCs, T cells from both healthy controls and AD patients produced increased levels of IFN- γ , IL-4 and IL-22 (Fig. 6). However, the expression levels of IL-4 and IL-22 were higher in cultures of DCs-T cells from AD patients than that of healthy controls (Fig. 6C and Supplementary Fig. 2).

4. Discussion

To our knowledge, the role of the allergen-DC-SIGN-DCs axis in AD has not been previously reported. Defective skin barrier functions in AD allow antigens to enter the skin, creating a milieu that shapes the immune response to the antigens [14–19]. DCs in skin can recognize invading pathogens and allergens [20–25] via pattern recognition receptors including CLRs. DC-SIGN is involved in the recognition and capture of many glycosylated antigens, including allergens and pathogens [26,27], by binding to their carbohydrate moieties, such as glucose, galactose, fucose [28] and N-acetylglucosamine. Binding of DC-SIGN by different ligands may result in different responses. The mechanisms include the affinity of the binding, the character of pathogen, and the co-receptor involved [10,14,18]. DC-SIGN binds with high affinity to fucosylated glycan structures that are generally believed to exist in a variety of allergens [10]. House dust mite and egg white exposure and sensitization pose a significant risk for AD development [29]. However, the mechanism is not clear, and the causal relationship among allergen exposure, allergic sensitization and AD remains to be established. Our data showed that expression of DC-SIGN on DCs was markedly increased in AD patients and was correlated with disease severity. DC-SIGN could bind to many allergens including Der p2 and Gal d2, which mediate the uptake of Der p2

and Gal d2 by Mo-DCs and induce Th2 and Th22 polarization. These results indicated that allergen-DC-SIGN-DCs axis may play an important role in AD initiation, maintenance and flare-up.

In the present study, we reported for the first time that there are more DC-SIGN-expressing DCs in the dermis of AD lesions compared with normal controls, and the expression level of DC-SIGN is higher than that of normal controls and was correlated with disease severity. In general, DC-SIGN expression is dependent on IL-4 signalling, and IL-13 may also participate in DC-SIGN induction [30]. The JAK-STAT signalling route is involved in IL-4-dependent induction of DC-SIGN. The transcription factor PU.1 also regulates basal and tissue-specific expression of DC-SIGN. The exact mechanism of the elevated expression of DC-SIGN in AD skin is not clear yet, and we infer that the micro-environment of AD skin might induce the expression of DC-SIGN by increased expression of IL-4 and IL-13. DC-SIGN is not only expressed on DCs but also on macrophages. Keratinocytes in epidermis also express substantial levels of DC-SIGN, especially under inflammatory conditions [31]. All of these DC-SIGN-expressing cells may participate in the pathogenesis of AD; however, the exact role of different cell types may differ from each other. A newly recognized DC-SIGN-expressing macrophage subset inhibited CD8 $^{+}$ T cell immunity and promoted CD4 $^{+}$ Foxp3 $^{+}$ Treg cell expansion upon binding by fucosylated ligands [32]. Keratinocytes might initiate an inflammatory response upon binding to antigens expressing the glucose motif.

We isolated and cultured Mo-DCs from AD patients and found that DC-SIGN was consistently expressed on the cells and mediated Der p2 and Gal d2 uptake. Activation of DCs by Der p2 and Gal d2 was shown by increased production of IL-6 and TNF- α , which was

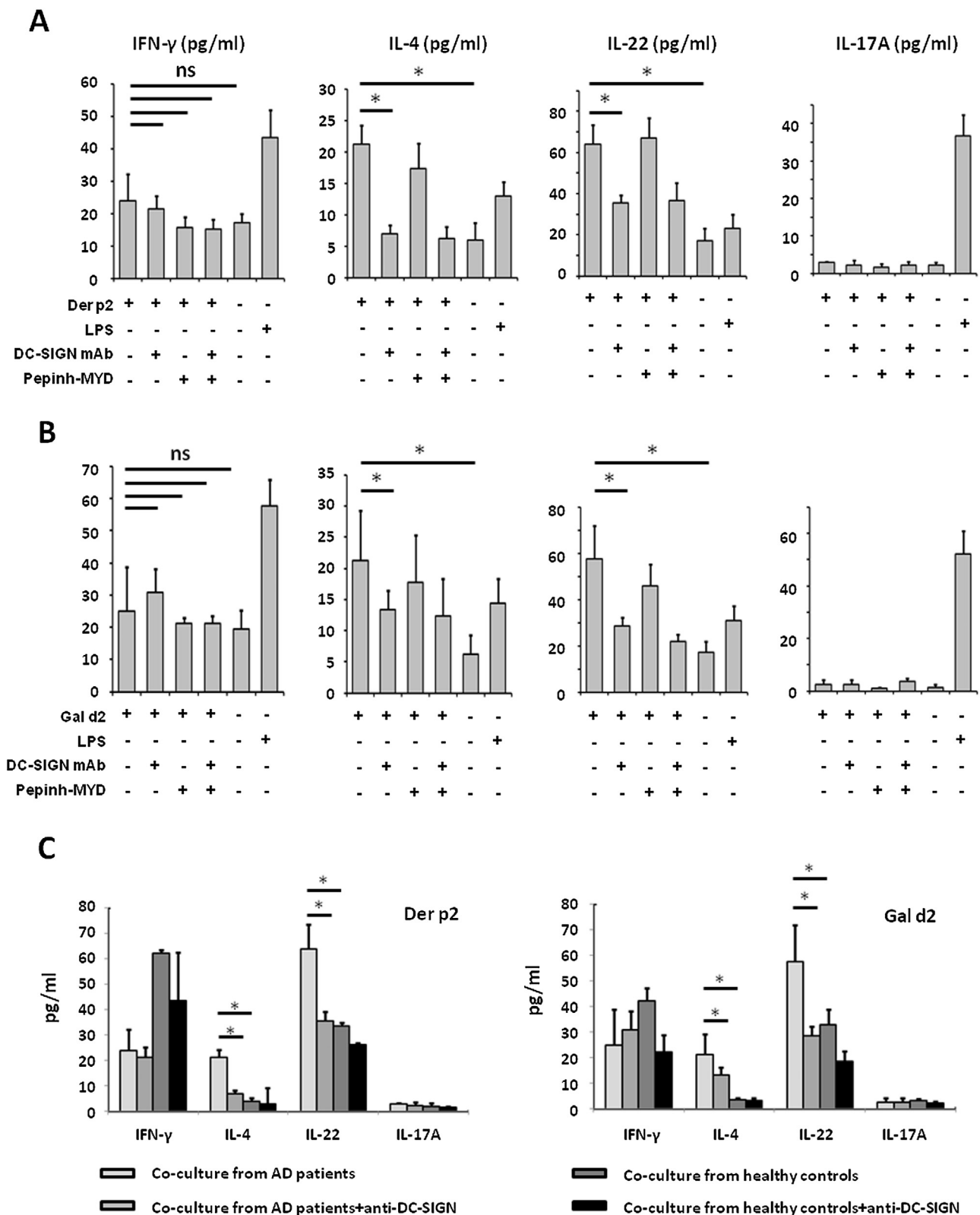


Fig. 6. Der p2 or Gal d2-exposed Mo-DCs induce naïve T cells towards Th2 and Th22 skewed phenotypes. (a and b) Mo-DCs from AD patients were co-cultured with naïve autologous T cells for 2 weeks with Der p2 or Gal d2. The supernatant was collected and subjected to IFN- γ , IL-4, IL-22 and IL-17A analysis by ELISA. DC-SIGN-specific blocking mAb was used to block the binding of Der p2 to DC-SIGN, and MyD88 inhibitory peptide was used to block the MyD88 signalling pathway. (c) Production of IFN- γ , IL-4, IL-22 and IL-17A by DCs-T cells cocultured from AD patients and healthy controls with Der p2 or Gal d2 were compared. Data represent results from at least three independent experiments and are expressed as the means $\pm$ S.E. * $P < 0.05$.

inhibited by DC-SIGN blocking Abs or Myd88 inhibitory peptide, suggesting a possible role of DC-SIGN and Myd88 signalling in Der p2 and Gal d2-induced DC activation. Previous studies showed that DC-SIGN signalling regulates TLR signalling [33]. Der p2 can bind to both DC-SIGN [34] and TLR4 [35]. DC-SIGN induces RAF1 phosphorylation, which modulates TLR induced NF- κ B activation. Upon TLR stimulation, the canonical NF- κ B subunit p65 is released from its inhibitor and translocates to the nucleus. Phosphorylated RAF1 induces p65 phosphorylation at Ser276 that functions as a binding site for the histone acetylase CBP. Acetylation of p65 induces enhanced and prolonged inflammatory factor transcription. Thus, blockade of either DC-SIGN or Myd88 signalling attenuates NF- κ B transcription and cytokine production; however, because DC-SIGN signalling exerts its effects on the Myd88 signalling pathway, there may be no additive effects when both an anti-DC-SIGN blocking antibody and Myd88 inhibitory peptide are used.

The fate of naïve T cell polarization is determined by the microenvironment in which the DCs reside. Previous data showed that in healthy donors, the presence of allergens, such as Der p1, dramatically enhanced Th1 polarization [25].

In AD patients, our study revealed that house dust mite and egg white allergens activate DCs via DC-SIGN and favour the priming of Th2 and Th22 responses. It has been recently reported that IL-1 β stimulation and NF- κ B signalling promoted the production of TSLP by Mo-DCs upon microbial stimulation [36], and it has also been reported that TNF- α and IL-6 could promote the differentiation of IL-22-producing T cells [37]. Our data showed that when cocultured with Der p2 or Gal d2, Mo-DCs produced elevated levels of TNF- α , IL-6 and IL-1 β . Thus, Der p2/Gal d2-stimulated Mo-DCs may also use a similar mechanism to produce TSLP and/or other Th2/Th22-skewing cytokines.

Based on the samples of AD patients, we discovered a novel regulatory pathway involving the functional interaction of house dust mite allergen or egg white allergen with DC-SIGN on DCs in the pathogenesis of AD. Our data demonstrated the existence of a functional allergen-DC-SIGN-DCs axis in controlling DC function and Th2/Th22 polarization, providing a foundation for further delineation of the pathogenic mechanisms of AD. Future studies are needed to examine the immunological consequences of allergen interactions with DC-SIGN on DCs and how this would contribute to the regulation of innate and adaptive immunity. Additionally, understanding the regulatory role of DC-SIGN in allergic diseases may offer an opportunity for the design of novel, safe and more efficacious therapies.

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Conflict of interest

The authors have no conflict of interest to declare.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.jdermsci.2016.08.008>.

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Table 1: Patient characteristics

	Study on cytokine expression		Studies on skin biopsy specimens		
	AD allergic to HDM	AD allergic to egg white	Mild AD	Severe AD	Normal controls
Subjects(n)	10	10	5	5	10
Mean age $\pm$ SD	30 $\pm$ 8.2219	34 $\pm$ 13.4090	30.4 $\pm$ 7.8294	30.8 $\pm$ 9.6799	31 $\pm$ 11.8415
SCORAD $\pm$ SD	33.51 $\pm$ 23.1957	30.49 $\pm$ 21.8607	13 $\pm$ 1.3491	63 $\pm$ 7.5954	Not applicable

Supplementary Figure 1.

Expression of TNF- α and IL-6 by Mo-DCs stimulated with Der p2 and Gal d2 in the presence of anti-DC-SIGN blocking antibody and isotype control antibody. Mo-DCs were incubated with Der p2 or Gal d2 for 24 h, and the supernatant was subjected for TNF- α (a) and IL-6 (b), analysis by ELISA. DC-SIGN-specific blocking mAb and isotype control were used to block the binding of Der p2 or Gal d2 to DC-SIGN. Data represent results from three independent experiments and are expressed as the mean $\pm$ S.E. * P <0.05.

Supplementary Figure 2.

Intracellular staining of IL-4 and IL-22 in CD4⁺ cells from coculture of MoDCs-Naïve T cells supplemented with Der p2/Gal d2. Mo-DCs from AD patients were co-cultured with naïve autologous T cells for 2 weeks with Der p2 or Gal d2. The cells were then subjected for intracellular staining with anti-IL-4 or anti-IL-22 antibody and analyzed by FACS. DC-SIGN-specific blocking mAb was used to block the binding of Der p2 to DC-SIGN, and MyD88 inhibitory peptide was used to block the MyD88 signalling pathway.

Supplementary Figure 3.

Expression of IL-10 by Mo-DCs stimulated with Der p2 and Gal d2. Mo-DCs from AD were incubated with Der p2 or Gal d2 for 24 h in the presence of anti-DC-SIGN blocking antibody or Pepinh-MyD, and the supernatant was subjected to IL-10 analysis by ELISA. Data represent results from three independent experiments and are expressed as the mean $\pm$ S.E. * P <0.05.

Supplementary Figure 4.

Mo-DCs from AD patients were co-cultured with naïve autologous T cells for 2 weeks with Der p2 or Gal d2. The supernatant was collected and subjected to IL-10 analysis by ELISA.. Data

represent results from three independent experiments and are expressed as the mean $\pm$ S.E.

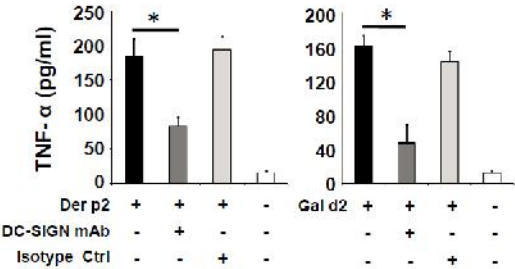
**P*<0.05.

Supplementary Figure 5.

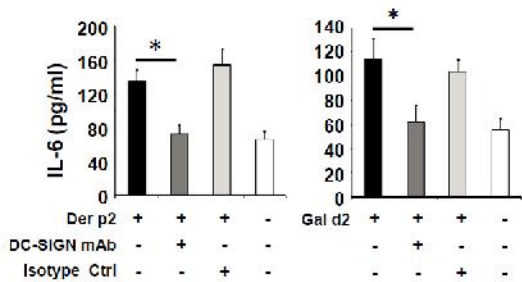
Proliferation of T cells in co-culture of Mo-DCs-Naïve T cells. Naïve T cells were stained with CFSE, and co-cultured with Der p2/Gal d2-stimulated Mo-DCs for 5 days. The cells were then labelled with anti-CD4 antibody and subjected to CFSE staining and analyzed by FACS.

Supplementary Figure 1

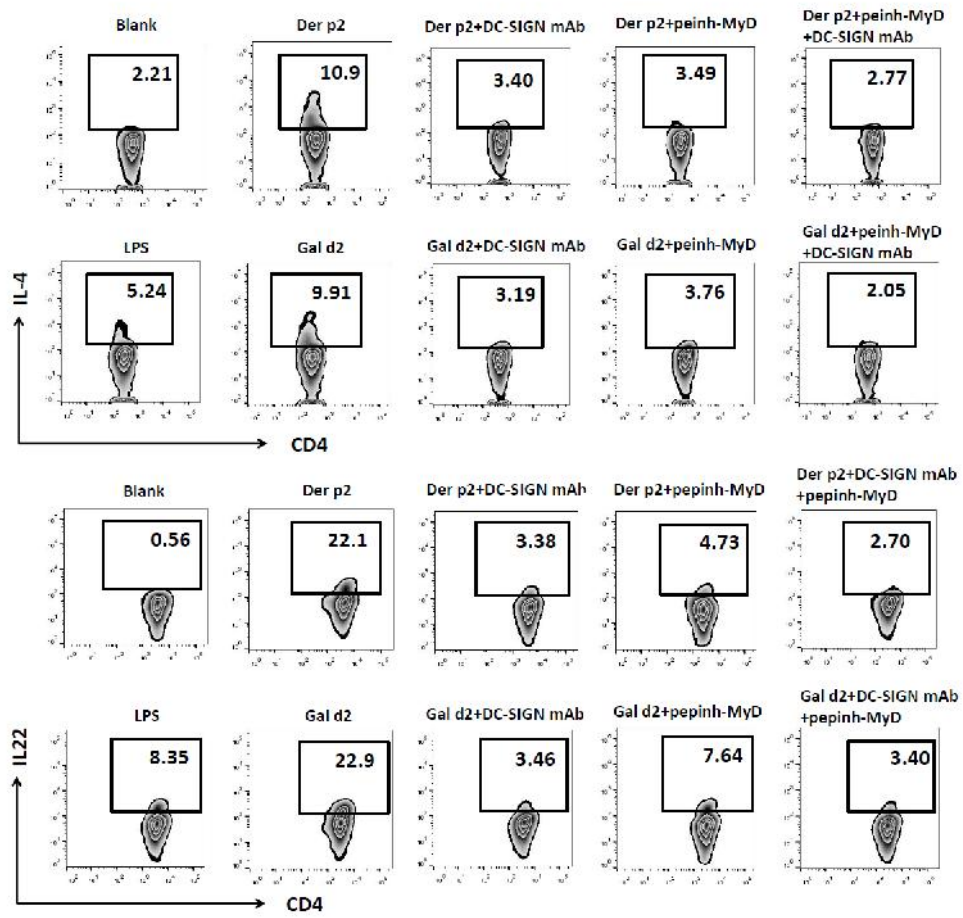
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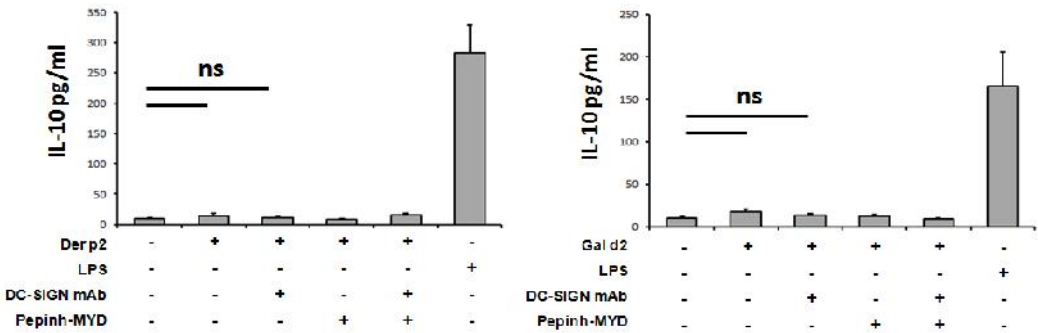
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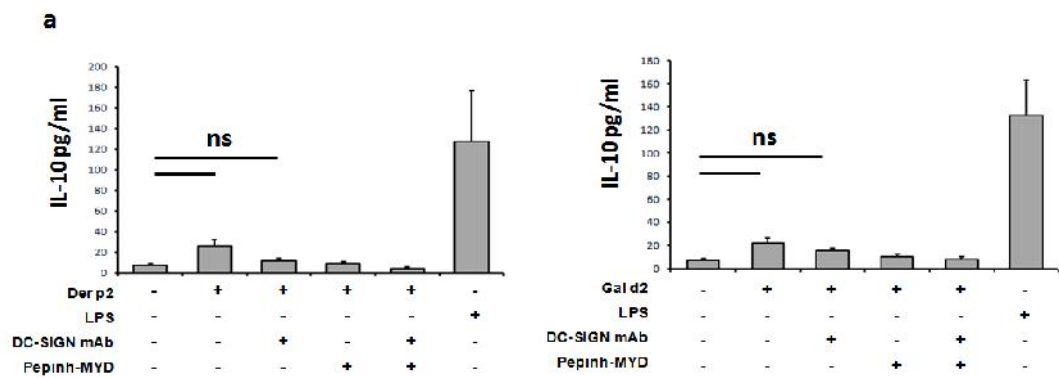
Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4



Supplementary figure 5

