The Major Glycoprotein Allergen from *Arachis hypogaea*, Ara h 1, Is a Ligand of Dendritic Cell-Specific ICAM-Grabbing Nonintegrin and Acts as a Th2 Adjuvant In Vitro

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The Major Glycoprotein Allergen from *Arachis hypogaea*, Ara h 1, Is a Ligand of Dendritic Cell-Specific ICAM-Grabbing Nonintegrin and Acts as a Th2 Adjuvant In Vitro

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Nonmammalian glycan structures from helminths act as Th2 adjuvants. Some of these structures are also common on plant glycoproteins. We hypothesized that glycan structures present on peanut glycoallergens act as Th2 adjuvants. Peanut Ag (PNAg), but not deglycosylated PNAg, activated monocyte-derived dendritic cells (MDDCs) as measured by MHC/costimulatory molecule up-regulation, and by their ability to drive T cell proliferation. Furthermore, PNAg-activated MDDCs induced 2- to 3-fold more IL-4- and IL-13-secreting Th2 cells than immature or TNF/IL-1-activated MDDCs when cultured with naive CD4+ T cells. Human MDDCs rapidly internalized Ag in a calcium- and glycan-dependent manner consistent with recognition by C-type lectin. Dendritic cell (DC)-specific ICAM-grabbing nonintegrin (DC-SIGN) (CD209) was shown to recognize PNAg by enhanced uptake in transfected cell lines. To identify the DC-SIGN ligand from unfraccionated PNAg, we expressed the extracellular portion of DC-SIGN as an Fc-fusion protein and used it to immunoprecipitate PNAg. A single glycoprotein was pulled down in a calcium-dependent manner, and its identity as Ara h 1 was proven by immunolabeling and mass spectrometry. Purified Ara h 1 was found to be sufficient for the induction of MDDCs that prime Th2-skewed T cell responses. Both PNAg and purified Ara h 1 induced Erk 1/2 phosphorylation of MDDCs, consistent with previous reports on the effect of Th2 adjuvants on DCs. *The Journal of Immunology*, 2006, 177: 3677–3685.
our understanding of Th2-biased responses to a wide variety of exogenous glycoproteins in predisposed individuals.

Materials and Methods

**Staphylococcus aureus** enterotoxin B (SEB), LPS, and human serum albumin were purchased from Sigma-Aldrich. Cholera toxin is from List Biological Laboratories. Human IL-1β, TNF-α, GM-CSF, and IL-4 were obtained from R&D Systems. PMA was obtained from Sigma-Aldrich. Brefeldin A and calcium ionophore, A23187, were obtained from Fisher Scientific. Solubil peanut Ag (PNAg) and purified Ara h 1 were prepared from defatted peanut flour. PNAg and purified Ara h 1 were determined to be endotoxin free (<0.01 EU/ml) by Limulus assay (Cambrex). Proteins were FITC conjugated using an amine-reactive succinimidyl fluorescein (Molecular Probes), according to the manufacturer’s protocol, with a 1:10 molar excess of FITC (or 30 mg of fluorescein per gram total protein in the case of heterogeneous PNAg). Molar ratios of FITC to protein were between 5 and 10:1 (or 1:1 for PNAg calculated using the m.w. of Ara h 1). This degree of labeling of PNAg was shown to not interfere with peanut-specific Ab binding of PNAg proteins (data not shown) and yet provides a sufficiently bright fluorescent signal for uptake studies. Chemically deglycosylated PNAg (dPNAg) was prepared by periodic acid treatment (100 mM (pH 7.2), 4°C, overnight) and diazoyzed against PBS before use. This treatment abolished PAS staining and increased mobility of glycoproteins present in PNAg (data not shown). Protein concentrations were determined by Bradford assay (Pierce Biotechnology).

**Activation assays**

Immature MDDCs were obtained from normal donors by positive selection of expression of CD14 from Ficoll-separated PBMC, according to the manufacturer’s protocol (EasySep; StemCell Technologies). These cells were cultured for 6 days in RPMI 1640 with 10% FCS supplemented with recombinant human GM-CSF and IL-4 (1000 and 800 U/ml, respectively). Medium was refreshed on day 3. On day 6, detached cells were harvested, washed, and counted. An aliquot of these day 6 cells was stained with a saturating mixture of previously titrated mAbs, including anti-CD3 (UCHT1), CD14 (M5E2), HLA-DR (G46-6) (or DQ; TU¨ 39), CD11c (B45-3), CD40 (SC5), CD83 (HB15e), and CD86 (2331) (all clones; BD Biosciences), for assessment of purity and differentiation to DC as well as baseline levels of expression of CD14 from Ficoll-separated PBMC, according to the manufacturer’s protocol (EasySep; StemCell Technologies). These cells were generally >90% class II CD11c+, <5% CD14+ (see Fig. 2, data for CD14 not shown). We typically obtained 3–6 × 10^5 DCs at day 6 starting from 70 to 100 × 10^5 PBMC. For MDDC activation, 5 × 10^5 cells per condition were stimulated with PNAg, as described, or controls (LPS, 100 ng/ml; cholera toxin, 1 μg/ml) for 48 h in RPMI 1640 with 10% FCS (HyClone). Cells were then harvested, washed, and fixed for assay of phenotype by flow cytometry. For some experiments, PNAg and controls were used in addition to medium with TNF-FIL-1 (10 ng/ml IL-1β and 50 ng/ml TNF-α). For plate-bound activation experiments, PNAg, dPNAg (100 μg), or LPS (1 μg) was incubated overnight in coating buffer (Sigma-Aldrich) in 96-well U-bottom plates. The wells were then washed, and MDDCs were added in complete medium for 48 h before staining as above for activation markers.

**Proliferation assays**

For measurement of DC activation by induction of naïve T cell proliferation, naïve T cells were washed and 5 × 10^5 were cultured in triplicate using 96-well U-bottom plates with increasing numbers of glycans or control-activated MDDCs (1:48, 1:24, 1:12, 1:6, or 1:3) irradiated DC to ratio either with or without 10 μg/ml SEB, as indicated, for each experiment. After 3 days, 1-H[3]Hthymidine was added overnight, and proliferation was measured as incorporation of radioactivity by scintillation counter. T cells and DCs alone (or with 10 μg/ml SEB) were also plated as controls for background proliferation, which was <100 cpm for all experiments (data not shown). Data were reproduced with several different normal donors’ DCs (n = 5).

**DC-T cell Th differentiation coculture**

MDDCs were obtained as above. Th1/Th2 skewing by activated DCs was assessed in coculture experiments with naïve T cells. For coculture experiments, 5 × 10^5 day 8 DCs (after 48-h stimulation with PNAg fractions or controls) were washed and recultured with 2 × 10^5 purified allogeneic CD4+CD45RA- T cells (naïve T cell selection kit (StemCell Technologies); cells were shown to be >95% CD4+CD45RA- by flow cytometry, data not shown). On day 5, cultures were supplemented with IL-2 (10 U/ml) and expanded as necessary. On day 14, when cells were rested, they were restimulated with PMA (5 ng/ml) and Ca2+ ionophore (250 ng/ml) for 4 h in the presence of brefeldin A (10 μg/ml) for accumulation of intracellular cytokine. These were then harvested, washed, stained with ethidium monoazide bromide (1 μg/ml; Molecular Probes) for staining of dead cells, then permeabilized and stained for a mixture of PE-conjugated (mAb) CD3 (H57-597), CD8 (53-6.7), CD45RA (H12-2F11), CD11c (HI digestion and cloning into cd5Lneg1. All constructs were confirmed by sequencing.

**EBNA 293 cells** were transfected by calcium phosphate precipitation, and secreted fusion protein was purified from cell culture supernatant using a protein A-agarose (Upstate Biotechnology) column. To identify DC-SIGN binding ligands from whole soluble PNAg, purified DC-SIGN-Fc was incubated in solution with unfraccionated PNAg in TBS with CaCl2 or EGTA for 2 h at 4°C. This was then applied to a protein A-agarose column and washed with 5 column volumes of TBS containing 0.1% Tween 20. Bound material was eluted by low pH with glycine, and fractions were analyzed by SDS-PAGE. The identity of DC-SIGN-bound Ara h 1 was confirmed by immunoblotting with specific mAb (clone 2F7; Indoor Bio-technologies) and confirmed by mass spectrometry peptide sequence analysis (Wistar Proteomics Facility) by >45 separate sequence matches to Ara h 1 in the sequence database SEQUEST.

ELISA was used to confirm specific DC-SIGN binding of Ara h 1. Microtiter plates (Nunc) were coated with 10 μg/ml Ara h 1, Ara h 2, PNAg, BSA, or coating buffer alone (NaHCO3; pH 9.6) overnight at 4°C. Non-specific binding was reduced by incubation with 2% v/v normal rabbit serum in PBS with 0.1% Tween 20. DC-SIGN-Fc or mutated DC-SIGN-Fc diluted in TBS with 10 mM CaCl2 or 2 mM EDTA was incubated for 1 h at room temperature, followed by detection by HRP-conjugated protein A (DakoCytomation) and substrate (SeeBlue-TMB; Kirkegaard & Perry Laboratories).

**Cell signaling and immunoblots**

One million day 6 MDDCs were serum starved for 2 h and then stimulated with indicated Ags or in the presence of 1% FBS containing medium with indicated Ags or control stimulus (500 μg/ml PNAg, 500 μg/ml dPNAg, 300 μg/ml Ara h 1, 300 μg/ml deglycosylated Ara h 1 (dAra h 1), 1 μg/ml LPS, 50 ng/ml TNF-α, and 10 ng/ml IL-1β). After indicated time points, cells were placed on ice and lysed in radioimmunoprecipitation assay buffer with rocking for 15 min at 4°C. Debris was pelleted in a precooled centrifuge and supernatants were subjected to SDS-PAGE.

For SDS-PAGE/Western blot immunoblotting experiments, 50 μg (Bradford protein assay; Pierce Biotechnology) of cell lysates was separated on 10% SDS gels and then transferred to polyvinylidine difluoride
Immobilon membrane. The membranes were dried after soaking in 100% methanol and then were blocked with 5% dry milk in TBST for 2 h at room temperature. Primary Abs (p-Erk 1/2, Erk 1/2, p-p38, p-SAK/JNK; all from Cell Signaling Technology) were diluted 1/1000 in TBST and incubated overnight at 4°C. Detection of primary Ab binding was by use of HRP-conjugated secondary Ab (Cell Signaling Technology) diluted 1/2000 in TBST, followed by chemiluminescence detection reagent (Amersham Biosciences). Semiquantitative analysis of results was conducted using the Quantity One software package (Bio-Rad).

Results

DCs are activated by binding PNAg glycan

It is recognized that in their role as pathogen receptors, some C-type lectin receptors not only facilitate Ag capture, but also participate in signaling DCs to become activated to elicit appropriate adaptive immunity (29, 30). We found that endotoxin-free soluble (data not shown) and immobilized PNAg, incubated with MDDCs for 48 h, induced up-regulation of surface HLA-DR, CD83, CD86, and CD40 in comparison with medium alone or periodate-treated PNAg (dPNAg; Fig. 1, A and B).

PNAg activation of MDDCs was further demonstrated by their ability to induce CD4⁺ T cell proliferation. Day 6 MDDCs were cultured for 48 h with medium, PNAg, or dPNAg. These were then washed, irradiated, and cocultured in replicates of decreasing DC numbers with 5 × 10⁴ allogeneic naive T cells to measure proliferation by thymidine incorporation. Proliferation of naive T cells was dose dependent on DCs, and those T cells cocultured with PNAg-activated DCs proliferated significantly more (Fig. 1C).

PNAg-activated DCs skew naive T cells toward a Th2 phenotype

We next tested the hypothesis that soluble PNAg activate DCs to preferentially prime naive CD4⁺ T cells toward a Th2 phenotype as measured by cytokine synthesis. We cultured day 6 MDDCs for 48 h with PNAg or controls, as above. These were then harvested, washed, and cultured with naive allogeneic CD4⁺ T cells (>90% purity) for 2 wk. Because it has been reported that immature DCs are Th2 skewing, we also tested whether PNAg stimulation induced a Th2-priming DC capacity even with strong activation. Therefore, we cultured day 6 MDDCs for 48 h with IL-1β and TNF-α (TNF/IL-1) and TNF/IL-1 plus PNAg or controls. There was no evidence of PNAg antagonism of TNF/IL-1 maturation (data not shown).

Example and summary data from three independent experiments comparing T cell phenotype by intracellular cytokine staining for IL-4, IL-13, and IFN-γ after coculture with PNAg-activated DCs vs DCs incubated with medium alone, TNF/IL-1, or control stimulants are shown in Fig. 2. Consistent with previous reports of Th2 induction by immature DCs, those DCs incubated with TNF/IL-1 induced lower percentages of IL-4- and IL-13-producing and higher percentages of IFN-γ-producing T cells (Fig. 2A). PNAg-activated DCs, however, induced on average twice as many IL-4- and IL-13-positive T cells in comparison with either medium (Fig. 2A) or TNF/IL-1 alone (Fig. 2, A and B). Summarized as the

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**FIGURE 1.** PNAg directly induces activation of MDDCs, and treatment with periodic acid reduces this activity. A, Open histograms represent unstimulated MDDCs vs filled histograms representing dPNAg-, PNAg-, or LPS-stimulated DCs, as indicated for each row; B, summary of independent experiments showing change in MFI relative to unstimulated MDDCs using plate-bound stimulants (n = 3; bars represent SD); C, PNAg-activated MDDCs induce stronger proliferation of naive T cells. A total of 5 × 10⁴ CD4⁺CD5RO⁻ T cells was cultured with decreasing numbers of irradiated allogeneic MDDCs that had been stimulated for 48 h with PNAg (Δ), dPNAg (V), or medium alone (■). Experiment shown is representative of five independent experiments.
change from TNF/IL-1-activated DCs, PNAg-activated DCs induced on average half the ratio of IFN-γ/H9253/IL-4/H11001 cells (Fig. 2C).

Soluble PNAg is rapidly internalized by myeloid DCs in a calcium- and carbohydrate-dependent manner

Preliminary experiments demonstrated that fluorescein-conjugated PNAg (FITC-PNAg) was avidly internalized by monocytes and CD11c+/H11001 myeloid, but not CD123+/H11001 plasmacytoid DCs (data not shown). Some C-type lectin receptors have evolved as pathogen receptors through recognition of nonmammalian glycan structures (29). We hypothesized that some member(s) of this family of receptors is likely to be involved in uptake of PNAg. To test this, we examined uptake of FITC-PNAg using in vitro MDDCs, which share many characteristics with immature myeloid DCs, including the expression of many C-type lectin receptors, such as macrophage mannose receptor, DC immunoreceptor, DC-SIGN, DEC-205, and Dectin-1 (29). CD11c+ immature MDDCs readily internalize FITC-PNAg, where it is visible in a punctate distribution consistent with endosomal localization (data not shown). By quantitative flow cytometry, uptake was reduced by ≥50% by the depletion of extracellular calcium or the presence of excess free mannan (Fig. 3A), consistent with the role of C-type lectin receptor-mediated uptake. Furthermore, chemically dPNAg uptake was reduced to a level comparable to that seen with calcium depletion or free mannan competition. There was no additive effect between mannan and EDTA, consistent with each inhibitor targeting the same pathway.

DC-SIGN is a receptor for PNAg glycan

We hypothesized that the C-type lectin receptor, DC-SIGN, may be an important receptor of peanut glycan, as it is exclusively expressed on myeloid DCs, has high affinity for nonmammalian fucosylated glycans, and has been implicated in Th2 immunity. To begin testing this hypothesis, we used a DC-SIGN-transfected cell line to compare uptake of fluorescein-labeled Ag. DC-SIGN-transfected Raji cells (a gift from D. Littman, New York University

FIGURE 2. PNAg-activated MDDCs induce naive T cell toward a Th2-skewed phenotype. A, Example of intracellular cytokine data from CD3+/CD4+/live T cells after 14-day coculture with PNAg-treated MDDCs showing increased IL-4- and IL-13-positive T cells compared with T cells cultured with MDDCs treated with medium or TNF-α/IL-1β; B, summary of four independent experiments with different donors showing fold change in percentage of positive cells for IFN-γ, IL-4, or IL-13, as indicated; C, same data expressed as a ratio of IFN-γ/IL-4 relative to control MDDCs. Error bars show SD.
were compared with untransfected Raji cells for their capacity to rapidly internalize FITC-PNAg and control Ags.

FITC-PNAg was taken up by DC-SIGN-transfected Raji more efficiently than in untransfected cells (Fig. 3B). Furthermore, this increased uptake of PNAg-FITC in the DC-SIGN-transfected cells was inhibited by mAb specific for DC-SIGN to a level comparable to that in untransfected Raji cells (Fig. 3C).

**DC-SIGN specifically binds the peanut glycoprotein allergen, Ara h 1**

To identify the DC-SIGN ligand from PNAg, we cloned the extracellular portion (aa 64–404), including the carbohydrate recognition domain, of DC-SIGN into the expression vector cd5Lneg1 (a gift from B. Seed), containing a CH2-CH3 fragment of human IgG1 that is bound with high affinity by protein A. This approach has been used previously to characterize the binding specificity of DC-SIGN (31).

This fusion protein was expressed in mammalian cells, purified using a protein A affinity column, and used to immunoprecipitate soluble PNAg in the presence or absence of calcium. A single band of 62–64 kDa bound to DC-SIGN-Fc in a calcium-dependent manner (Fig. 4A, lanes 1 and 2). This proved by immunoblot analysis (Fig. 4A, lanes 3 and 4) to be reactive with mAb specific for the major glycoprotein allergen of peanut, Ara h 1. Identification was also confirmed by in gel trypsin digestion/peptide mass spectrometry (data not shown).

To determine whether binding to Ara h 1 was consistent with previous structural data of the carbohydrate binding domain of DC-SIGN (32), we also constructed a mutated DC-SIGN-Fc protein with substitutions of N365D and D366A, two residues shown to be critical for calcium coordination and carbohydrate binding (33, 34). An ELISA was used to test binding. Wild type, but not the mutated DC-SIGN, bound both Ara h 1 and PNAg in the presence of calcium (Fig. 4B), but not EDTA. There was no specific binding of Ara h 2 or bovine albumin controls.

**Ara h 1-activated DCs prime a Th2 response in a glycan-dependent manner**

If Ara h 1-DC-SIGN interactions are important for the DC activation measured with PNAg, purified Ara h 1 may be sufficient for inducing this response. The effect of purified Ara h 1 on DC maturation as measured by up-regulation of class II and costimulatory molecule expression or by allostimulatory activity was more modest and inconsistent than the activity of whole PNAg (data not shown). However, Ara h 1-activated DCs were capable of inducing Th2 skewing of naive T cells (Fig. 5). Day 6 MDDCs were cultured with medium or TNF-α/IL-1 alone vs with Ara h 1 or controls. In comparison with either medium alone (data not shown) or TNF-α/IL-1β (Fig. 5), Ara h 1-stimulated DCs induced 2.5- to 3-fold more IL-4- and IL-13-positive T cells. Furthermore, the percentage of IFN-γ-positive CD4 T cells was lower when naive T cells were cultured with Ara h 1-activated DCs. Chemically dAra h 1 had no effect. DCs cultured with another peanut glycoprotein, Ara h 2, also had no effect on Th differentiation. There was no increase in the percentage of IL-10-secreting CD4 T cells induced by Ara h 1-activated DCs (Fig. 5A).

**Unfractionated PNAg and purified Ara h 1 activate MAPK Erk 1/2**

Recently, Agrawal et al. (35–37) demonstrated that Th2 PAMPs, including schistosome egg glycoproteins (SEA), which are known to be bound by DC-SIGN, preferentially activate Erk 1/2 over p38...
and JNK, and that this leads to stabilization of c-Fos and suppression of IL-12. Because PNAg, like SEA, is a ligand for DC-SIGN and induces a Th2-skewing DC phenotype, we hypothesized that PNAg may have a similar effect on MAPK activation.

To test this, day 6 MDDCs were incubated for short times with PNAg or dPNAg and controls, followed by cell lysis and immunoblotting for total and phosphorylated Erk 1/2, JNK, and p38. Consistent with the proposed mechanism of DC activation by SEA, phospho-Erk 1/2 is increased 3-fold as a ratio to total Erk by incubation with PNAg, while JNK and p38 are not detectably activated (Fig. 6A).

Finally, we looked to see whether Ara h 1, like whole PNAg, induces activation of Erk. MDDCs were cultured with purified Ara h 1 or dAra h 1 for 2, 5, 10, or 20 min, and assayed for phosphorylation of MAPKs by immunoblot. Ara h 1 induced strong Erk 1/2 phosphorylation by 2 min, which is sustained at least until 20 min. In contrast, dAra h 1 induced a weaker response (Fig. 6B).

Discussion
Previous studies have identified the major allergen, Ara h 1, as a glycoprotein with one predicted N-glycosylation site (38). Kolarich and Altmann (18) recently described the major glycan species of Ara h 1 as Man(3(-4))XylGlcNAc(2), a complex glycan containing a β1-2 xylose attached to the proximal mannose of the glycan core. This structure, as well as complex glycans containing an α1-3 fucose residue attached to the proximal N-acetylgalactosamine of the chitobiose core, have not been reported from mammalian glycoproteins, although they are common in plants, mollusks, and arthropods (21, 22). Individuals with IgE-mediated allergy to bee venom and plant pollen or foods have been shown...
to make specific IgE to these structures (25–27). Bardor et al. (28) also found that 25–50% of nonallergic individuals made a humoral immune response to these epitopes.

Core α1-3 fucose and β1-2 xylose residues are also common on glycoproteins of schistosomes and other helminths (39), and have been identified as part of the humoral response in helminth parasite infection (40, 41). In addition to their role as specific epitopes, evidence increasingly demonstrates that some schistosome glycans modulate the immune response (12, 14, 15). Faveeuw et al. (12) showed that α1-3 fucose- and β1-2 xylose-containing complex glycans derived from schistosome egg Ags are potent inducers of Th2 immune responses. Several defined α1-3 fucose-bearing glycoconjugates have subsequently been identified as conferring at least some of this activity (13, 15, 42, 43).

C-type lectin receptors are a very large family of proteins named for their calcium-dependent carbohydrate binding. These are evolutionarily ancient molecules that have evolved in part as pathogen receptors, although many also have endogenous ligands. DC-SIGN is an ITAM-containing type II member of the C-type lectin family that has been identified as a receptor for schistosome glycoproteins as well as a number of pathogens associated with suppression of Th1 and/or enhancement of Th2 immunity (30, 31, 43–48).

We have now shown evidence that glycans present in an aequous extract of peanut facilitate Ag uptake and activation of DCs in a manner that favors the priming of Th2 responses. The effect of PNAg on DCs is not due to contamination with endotoxin as all Ag preparations were shown to be endotoxin free. The involvement of glycan/C-type lectin receptor(s) was suggested by the inhibition of Ag uptake by both extracellular calcium depletion and excess free
mannot and the loss of binding and activity by periodate treatment of Ag.

DC-SIGN, which is expressed exclusively on myeloid DCs, recognizes a ligand present in peanut extract. That ligand has proved to be the major peanut glycoprotein Ag, Ara h 1. The recognition of Ara h 1 is calcium dependent and periodate sensitive, features consistent with specific recognition of Ara h 1 glycan. Furthermore, site-specific mutation of DC-SIGN at the primary carbohydrate-binding site abolishes Ara h 1 recognition.

Ara h 1 alone was sufficient to activate DCs to prime naïve T cells toward a Th2-biased phenotype. Treatment of DCs with both unfractionated PNAg and Ara h 1 induces Erk 1/2 phosphorylation, which is likely to be involved in the induction of currently undefined DC gene expression favoring Th2 induction. Pulendran and colleagues (35–37) have shown that induction of a Th2-priming MDDC phenotype by several stimulants including two that interact with ITAM-containing C-type lectin receptors (zymosan via Dectin-1 and SEA via DC-SIGN) involves Erk phosphorylation and stabilization of c-Fos. One of the most proximal signaling events following Dectin-1 binding is recruitment of Syk (11, 49). We hypothesize that DC-SIGN, via Syk recruitment and activation via its ITAM, modulates DC gene expression in a similar manner. Preliminary experiments with the semiselective Syk inhibitor, piceatannol, have demonstrated inhibition of PNAg and Ara h 1 uptake (data not shown), as has been shown for Dectin-dependent zymosan uptake (11).

In contrast to other data on DC response to the DC-SIGN ligands mycobacterial ManLam (47) and SEA (35), or to the Dectin-1 ligand zymosan (36), neither PNAg nor Ara h 1 alone induces IL-10 production from MDCCs either with or without the additional stimulus of CD40L (data not shown). This difference may be due to the integration of several signaling pathways, including co-signaling with TLRs in the response to zymosan, SEA, or ManLam. For example, Thomas et al. (50) have shown that the full response to SEA is TLR4-dependent and zymosan is known to signal through TLR2 in concert with Dectin-1 (36, 51–54). We have not yet examined the ability of Ara h 1 to antagonize or modulate the response to LPS or other innate stimuli other than TNF/IL-1.

There are a number of potential mechanisms of DC influence on T cells to be investigated, including differential expression of Notch ligands, DC-T cell signal duration or strength, and/or co-stimulatory molecule expression (55–57). The absence of a clear and consistent DC phenotype associated with Th2 priming that would be analogous to IL-12 production for Th1 priming has led to the hypothesis that Th2 responses are the result of a default response. Potentially consistent with this, many Th2 adjuvants appear to have weak effects on the activation/maturatation phenotype of DCs. However, several experimental systems have produced data that separate DC activation, as defined by up-regulation of class II or induction of proliferation, from their ability to direct Th1 or Th2 phenotype (58). In this study, we have shown that PNAg and Ara h 1 activation of DCs, with or without the addition of IL-1β and TNF-α to induce a mature phenotype, is Th2 skewing and therefore most likely represents a gain-of-function response. A recent study using the OVA-specific TCR transgenic strain, DO11.10, to isolate adjuvant effects of parasite infection when looking at the immune response to OVA, supported the role of OX40L in the production of Ag-specific IL-4 (59). Identification of the DC responses to Ara h 1 that confer Th2-priming activity is an area of current investigation.

Ara h 1 is the most abundant glycoallergen present in unfractionated PNAg, comprising ~10% of the total protein content. The finding that a plant glycoallergen is a ligand of DC-SIGN and can act directly to activate DCs to prime Th2 responses is likely to be of broad significance as glycans identified in peanut are ubiquitous among plant glycoproteins. Additional features of Ara h 1, such as its resistance to proteolysis, may act in concert with the activity of N-glycans to promote its allergenicity. Ara h 1, like other vicilins, forms trimers, and this quaternary structure has been reported to enhance its allergenicity (60). This structure may also result in an increased valency of glycan-lectin interactions.

DC-SIGN recognition of nonmammalian glycans present in plants seems most likely to represent the unintended consequence of its role in pathogen recognition. By enhancement of Ag uptake and activation of DCs, the presence of similar glycan PAMPs from insects, crustaceans, and mollusks suggests that glycoallergens from these sources may also act as Th2 adjuvants that contribute to allergic sensitization in susceptible individuals.

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Disclosures
The authors have no financial conflict of interest.

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