

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 Vitro¹

Wayne G. Shreffler,^{2*} Russell R. Castro,* Z. Yesim Kucuk,* Zachary Charlop-Powers,* Galina Grishina,* Steven Yoo,* A. Wesley Burks,[†] and Hugh A. Sampson*

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 unfractionated 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.

Much of the research on allergen immunogenicity has focused on the structural properties, such as size, solubility, stability, etc., that contribute to their access to the mucosal-associated immune compartment (1–3). There has been attention more recently to whether these proteins, or associated molecules, possess innate immunostimulatory properties (4–9). Allergens, whether present in airborne pollen, arthropod feces, or in the diet, are encountered by the mammalian immune system as part of complex conglomerations of protein, carbohydrate, and lipid. Just as the mammalian immune system has evolved mechanisms to recognize bacterial proteins in association with pathogen-associated molecular patterns (PAMPs)³ that induce appropriate Th1 responses (10, 11), part of what makes a protein allergenic may be determined by the context in which it is encountered; some molecular patterns have Th2 adjuvant function.

Among the well-studied Th2 PAMPs are *N*-glycans from *Schistosoma mansoni* egg Ags (12–15). Core α -fucosylated and β -xy-

losylated complex mannose glycans implicated from schistosomes are common on plant (16, 17), arthropod (18–20), and helminth, but not mammalian (21, 22) glycoproteins. Therefore, these glycans make attractive candidates as non-self structures recognized by the innate immune system to induce Th2-skewed responses that contribute to clinical allergy in susceptible individuals.

Allergy to peanut (*Arachis hypogaea*) tends to be persistent and severe, and it represents an important public health problem of increasing prevalence (23). There are numerous glycoproteins present in soluble peanut extract, and they have been shown to bear complex nonmammalian *N*-glycans (18, 24). The role of these structures as B cell and even T cell epitopes is well known (25–28).

We hypothesized that peanut glycans play a role in enhancing the Th2 immunogenicity of peanut allergens. To investigate this, we examined the effect of unfractionated and purified peanut allergens on the activation of normal donor monocyte-derived dendritic cells (MDDCs) as measured by their ability to induce naive T cell proliferation and differentiation as well as the up-regulation of markers of maturation and activation. To determine the necessity of glycans, we used periodic acid treatment to degrade carbohydrate structures. Evidence of periodate-sensitive activity led us to test for the involvement of C-type lectin receptors generally by showing dependence on extracellular calcium for binding and competitive inhibition with free mannan. Dendritic cell (DC)-specific ICAM-grabbing nonintegrin (DC-SIGN) involvement was specifically tested for by examining Ag uptake in a transfected cell line. We then constructed a DC-SIGN-Fc fusion protein to directly identify the DC-SIGN ligand. Finally, we tested whether this ligand, Ara h 1, is sufficient for activating DCs.

The finding that DC-SIGN directly binds a major glycoallergen that activates DCs to prime Th2 responses is novel and places the response to allergens in an evolutionary context that has broad implications for

*Jaffe Food Allergy Institute, Department of Pediatrics, Division of Allergy and Immunology, Mount Sinai School of Medicine, New York, NY 10029; and [†]Department of Pediatrics, Division of Allergy/Immunology, Duke University, Durham, NC 27706

Received for publication March 27, 2006. Accepted for publication June 15, 2006.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¹ W.G.S. was supported by National Institutes of Health Grant K12HD052890 and the Food Allergy Initiative.

² Address correspondence and reprint requests to Dr. Wayne G. Shreffler, Department of Pediatrics, Box 1198, Division of Allergy and Immunology, Mount Sinai Medical Center, One Gustave L. Levy Place, New York, NY 10029. E-mail address: wayne.shreffler@mssm.edu

³ Abbreviations used in this paper: PAMP, pathogen-associated molecular pattern; dAra h 1, deglycosylated Ara h 1; MDDC, monocyte-derived dendritic cell; DC, dendritic cell; DC-SIGN, DC-specific ICAM-grabbing nonintegrin; dPNAg, deglycosylated peanut Ag; MFI, mean fluorescence intensity; PNAg, peanut Ag; SEA, schistosome egg glycoprotein; SEB, *Staphylococcus aureus* enterotoxin B.

our understanding of Th2-biased responses to a wide variety of exogenous glycoproteins in predisposed individuals.

Materials and Methods

Reagents

Staphylococcus aureus enterotoxin B (SEB), LPS, and human serum albumin were purchased from Sigma-Aldrich. Cholera toxin is from List Biological Laboratories. Human rIL-1 β , 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. Soluble 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/mg) by *Limulus* assay (Cambrex). Proteins were FITC conjugated using an amine-reactive succinimidyl fluorescein (Molecular Probes), according to the manufacturer's protocol, with a 10:1 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 dialyzed 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; Tü39), CD11c (B-ly6), CD40 (5C3), CD83 (HB15e), and CD86 (2331) (all clones; BD Biosciences), for assessment of purity and differentiation to DC as well as baseline maturation. Fluorescence data were acquired on a modified LSR-II running Diva 4.0 (BD Immunocytometry Systems). These cells were generally >90% class II⁺ CD11c⁺, <5% CD14⁺ (see Fig. 2, data for CD14 not shown). We typically obtained 3–6 $\times 10^6$ DCs at day 6 starting from 70 to 100 $\times 10^6$ PBMC. For MDDC activation, 5 $\times 10^4$ cells per condition were stimulated with PNAg, as described, or controls (LPS, 100 ng/ml; cholera toxin, 1 μ g/ml) for 48 h in RPMI 1640 with 10% FCS (HyClone). Cells were then harvested, washed, and fixed for assessment of phenotype by flow cytometry. For some experiments, PNAg and controls were used in addition to medium with TNF/IL-1 (10 ng/ml IL-1 β and 50 ng/ml TNF- α). For plate-bound activation experiments, PNAg, dPNAg (100 μ g), or LPS (1 μ g) 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 naive T cell proliferation, naive T cells were washed and 5 $\times 10^4$ were cultured in triplicate using 96-well U-bottom plates with increasing numbers of glycan or control-activated MDDCs (1:48, 1:24, 1:12, 1:6, or 1:3 irradiated DC to T ratio) either with or without 10 pg/ml SEB, as indicated, for each experiment. After 3 days, [³H]thymidine was added overnight, and proliferation was measured as incorporated radiation by measurement by scintillation counter. T cells and DCs alone (or with 10 pg/ml SEB) were also plated as controls for background proliferation, which was <100 cpm for all experiments (data not shown). Data shown 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 naive T cells. For coculture experiments, 5 $\times 10^4$ day 8 DCs (after 48-h stimulation with PNAg fractions or controls) were washed and recultured with 2 $\times 10^5$ purified allogeneic CD4⁺CD45RA⁺ T cells (naive 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 μ g/ml) for accumulation of intracellular cytokine. These were then harvested, washed, stained with ethidium monoazide bromide (1 μ g/ml; Molecular Probes) for staining of dead cells, then fixed, and permeabilized for staining with a mixture of previously titrated mAbs against CD3 (UCHT1), CD4 (RPA-T4), IL-4 (3010.211), IL-13 (JES10-5A2), IL-2 (JES6-5H4), and IFN- γ (B27) (all obtained from BD Biosciences). Single-color compensation samples and fluorescence minus one gating control samples were prepared using anti-mouse Ig beads (Bangs Laboratories) and aliquots of stimulated cells, respectively. Fluorescence data were acquired and autocompensated on a modified LSR-II configured for seven-color parameters using FACS Diva software (version 4.0; BD Biosciences). Offline analysis was done using FlowJo version 6.0 (Tree Star). We acquired a minimum of 10⁵ live (ethidium monoazide bromide⁻) CD3⁺CD4⁺ events, allowing sufficient power to discriminate differences of 0.3%.

Ag uptake

After washing and resuspending in complete medium, 10⁵ MDDCs or Raji cells were incubated with optimal concentrations of FITC-conjugated glycoprotein (optimized for each Ag, between 10 and 200 μ g/ml) for 30 min at 37°C. Uptake is measured as either FITC mean fluorescence intensity (MFI) or fold increase compared with untreated identical cells. At least 10⁴ live cells defined by forward and orthogonal light scatter and exclusion of propidium iodide (0.5 μ g/ml; BD Pharmingen) were acquired. For anti-DC-SIGN-blocking experiments, cells were incubated 10 min before addition of Ag with increasing concentrations of anti-DC-SIGN (clone 120507; R&D Systems).

DC-SIGN fusion proteins and binding assays

The sequence containing the extracellular C terminus of DC-SIGN (residues 64–404) was amplified from DC-SIGN-transfected Raji cells (a gift from D. Littman, New York University, New York, NY) and cloned into *NheI/BamHI*-digested cd5Lneg1 (a gift from B. Seed, Massachusetts General Hospital, Boston, MA) in frame with the CD5 leader peptide as well as a human IgG1 fragment containing the C_H2 and C_H3 domains. Mutant DC-SIGN with N365D and D366A substitutions was constructed by PCR site-directed mutagenesis, and the product was TA cloned into pCR-TOPO II (Invitrogen Life Technologies) before *NheI/BamHI* 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 unfractionated 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 Biotechnologies) 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 μ g/ml Ara h 1, Ara h 2, PNAg, BSA, or coating buffer alone (NaHCO₃; pH 9.6) overnight at 4°C. Nonspecific 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 TBST 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 μ g/ml PNAg, 500 μ g/ml dPNAg, 300 μ g/ml Ara h 1, 300 μ g/ml deglycosylated Ara h 1 (dAra h 1), 1 μ g/ml LPS, 50 ng/ml TNF- α , and 10 ng/ml IL-1 β). 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 collected for further analysis.

For SDS-PAGE/Western blot immunoblotting experiments, 50 μ g (Bradford protein assay; Pierce Biotechnology) of cell lysates was separated on 10% SDS gels and then transferred to polyvinylidene difluoride

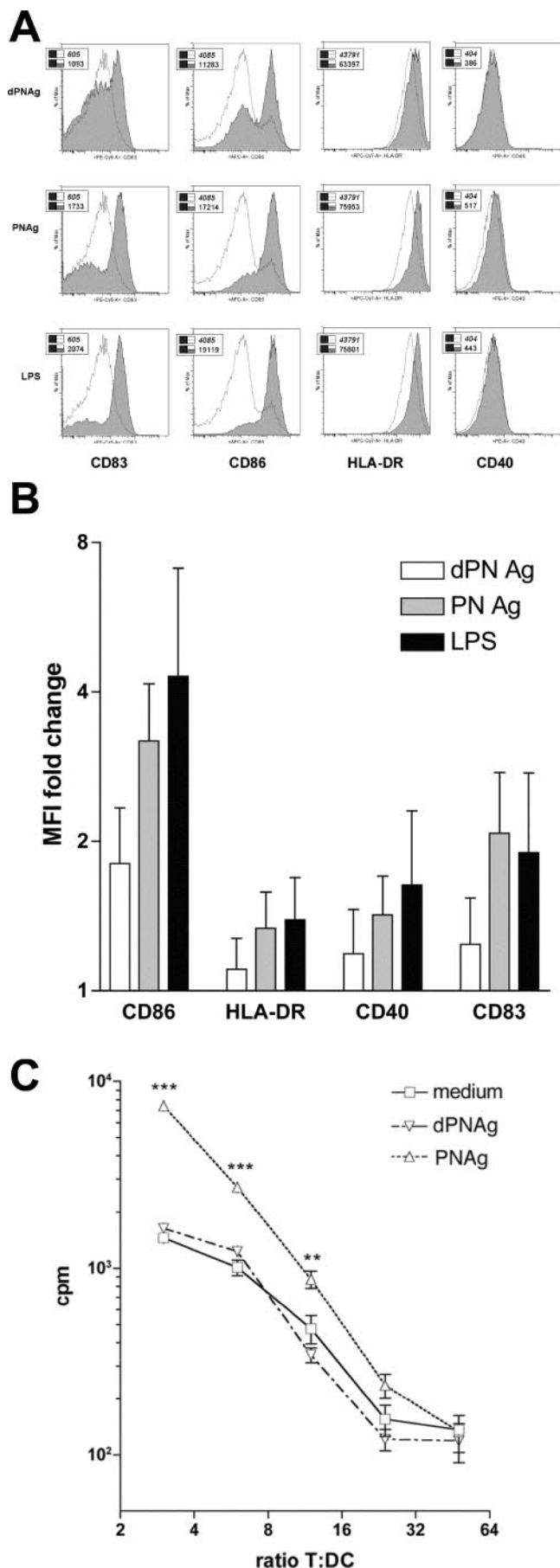


FIGURE 1. PNAg directly induces activation of MDDCs, and treatment with periodic acid reduces this activity. *A*, Open histograms represent un-

immobilized 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 naive 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 5×10^4 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-1 β 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

stimulated 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 5×10^4 CD4⁺CD45RO⁺ T cells was cultured with decreasing numbers of irradiated allogeneic MDDCs that had been stimulated for 48 h with PNAg (Δ), dPNAg (∇), or medium alone ($\square$). Experiment shown is representative of five independent experiments.

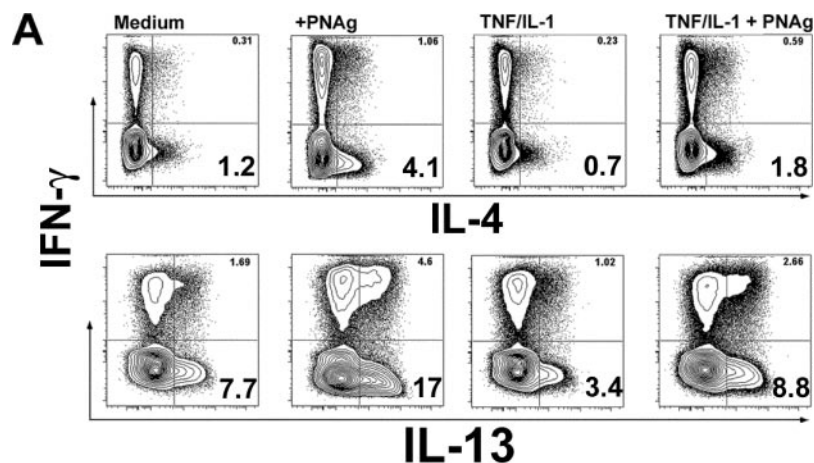
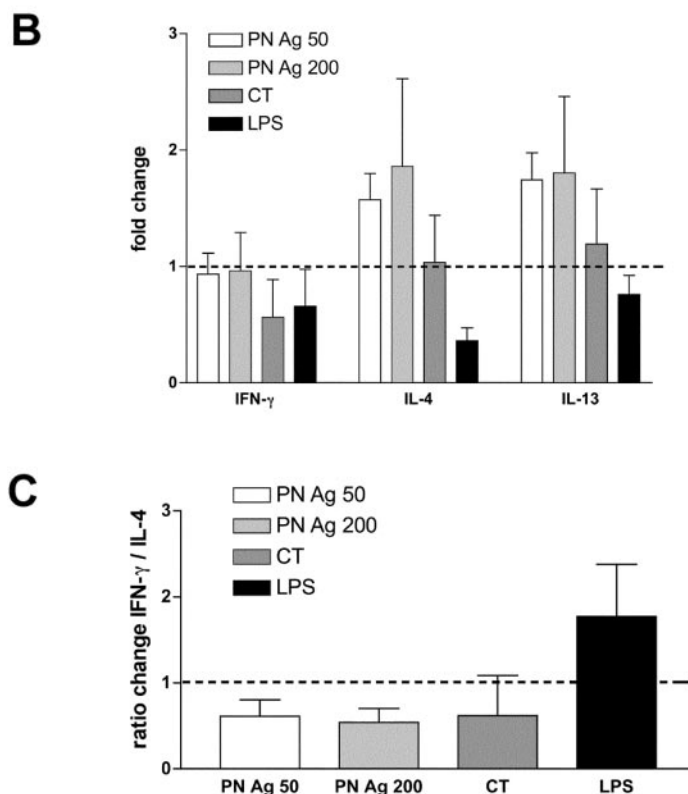


FIGURE 2. PNAg-activated MDDCs induce naive T cell toward a Th2-skewed phenotype. *A*, Example of intracellular cytokine data from CD3⁺CD4⁺ live T cells after 14-day coculture with PNAg-treated MDDCs showing increased IL-4- and IL-13-positive T cells compared with T cells cultured with MDDCs treated with medium or TNF- α /IL-1 β ; *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.



change from TNF/IL-1-activated DCs, PNAg-activated DCs induced on average half the ratio of IFN- γ ⁺/IL-4⁺ 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⁺ myeloid, but not CD123⁺ 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

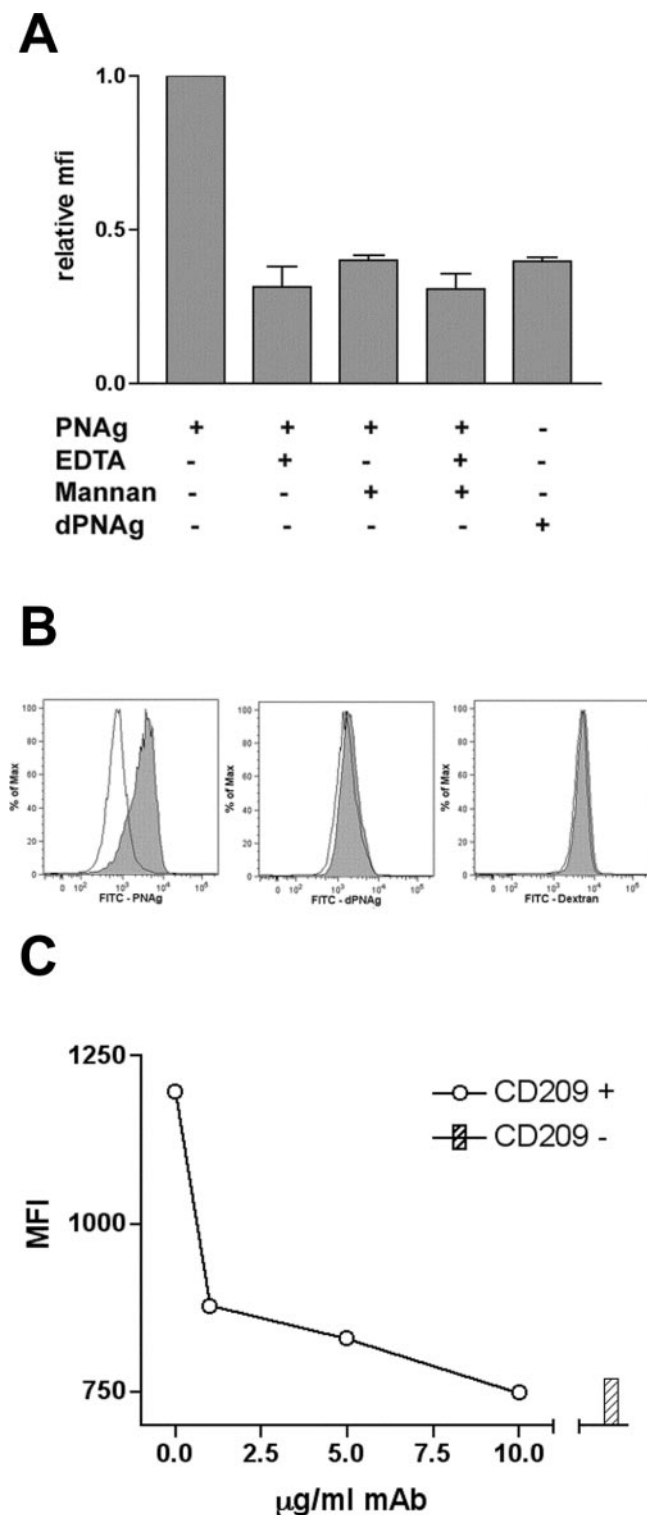


FIGURE 3. PNAg uptake is calcium and glycan mediated, and DC-SIGN binds a ligand from PNAg. *A*, Uptake of PNAg-FITC by MDDCs in the presence of EDTA to deplete extracellular Ca^{2+} or with excess free mannan quantified as MFI relative to untreated MDDCs. Uptake of periodate-treated PNAg (dPNAg) is shown for comparison; *B*, CD209-transfected Raji cells (filled histograms) preferentially internalize PNAg (left), but not dPNAg (middle) or dextran (right) in comparison with untransfected Raji (open histograms); *C*, enhanced uptake of PNAg-FITC, measured as MFI, is blocked by mAb to DC-SIGN (○) to a level comparable to that seen in untransfected Raji (◼).

Skirball Institute, New York, NY) 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. 3*B*). 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. 3*C*).

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 $\text{C}_{\text{H}2}\text{-C}_{\text{H}3}$ 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. 4*A*, lanes 1 and 2). This proved by immunoblot analysis (Fig. 4*A*, 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. 4*B*), but not EDTA. There was no specific binding of Ara h 2 or bovine albumin controls.

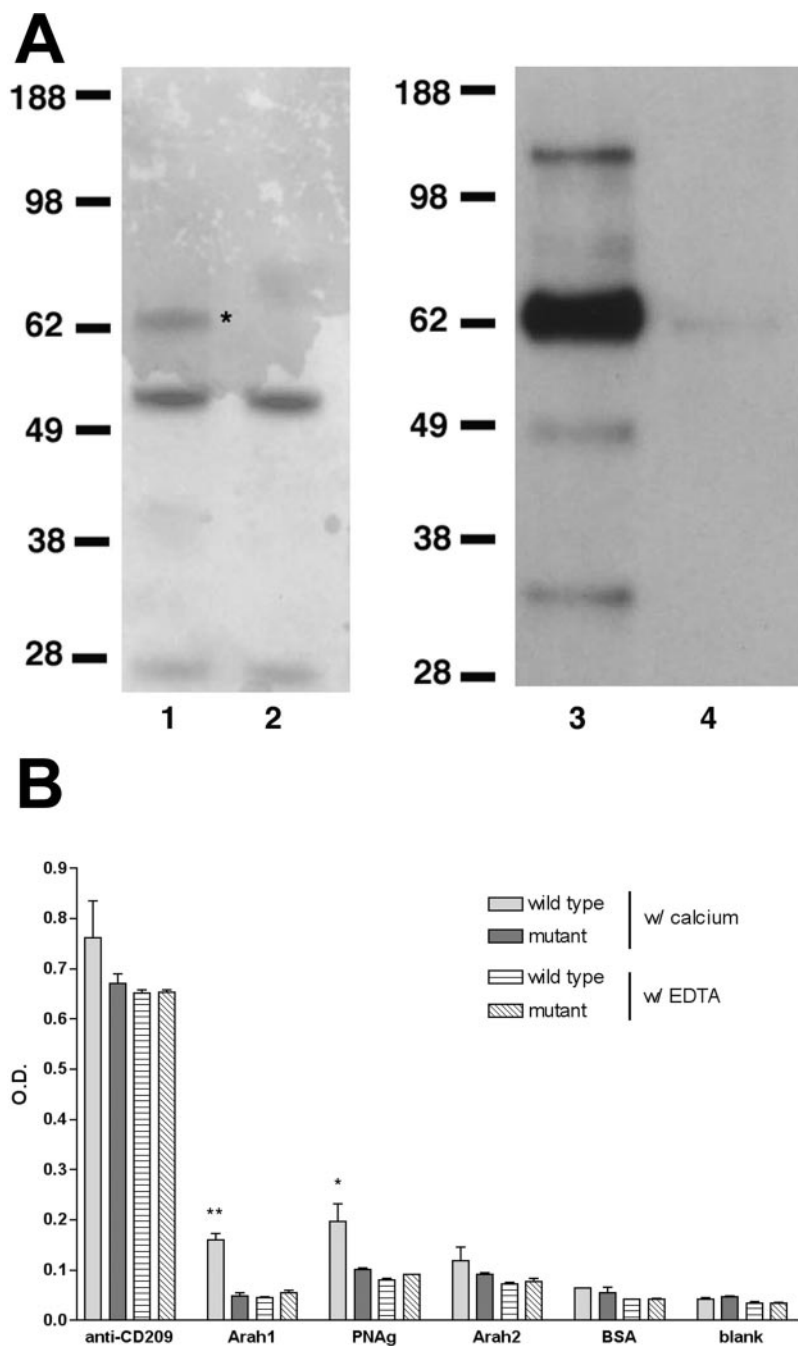
Ara h 1-activated DCs prime a Th2 response in a glycan-dependent manner

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 $\text{TNF-}\alpha/\text{IL-1}\beta$ alone vs with Ara h 1 or controls. In comparison with either medium alone (data not shown) or $\text{TNF-}\alpha/\text{IL-1}\beta$ (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. 5*A*).

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

FIGURE 4. DC-SIGN binds Ara h 1 from PNAg in a Ca^{2+} -dependent manner. **A**, Lanes 1 and 2, SDS-PAGE of DC-SIGN-Fc/protein A column-bound material showing a ~62-kDa band (*) present from binding experiment done with calcium (lane 1), but not in the presence of EGTA (lane 2). Additional bands are bovine IgG from DC-SIGN-Fc-containing culture supernatant. Lanes 3 and 4, show the same precipitates immunoblotted with anti-Ara h 1 mAb; **B**, indicated Ags were coated to ELISA plates and then incubated with Fc fusion proteins containing either the extracellular portion of DC-SIGN (wild type) or the extracellular portion mutated at N365 and D366 (mutant), two sites previously shown to be critical for carbohydrate binding. **, $p < 0.01$; *, $p < 0.05$ for comparison between wild type and mutant by *t* test. Error bars show SEM.



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 β 1-2 xylose attached to the proximal mannose of the glycan core. This structure, as well as complex glycans containing an α 1-3 fucose residue attached to the proximal *N*-acetylglucosamine 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

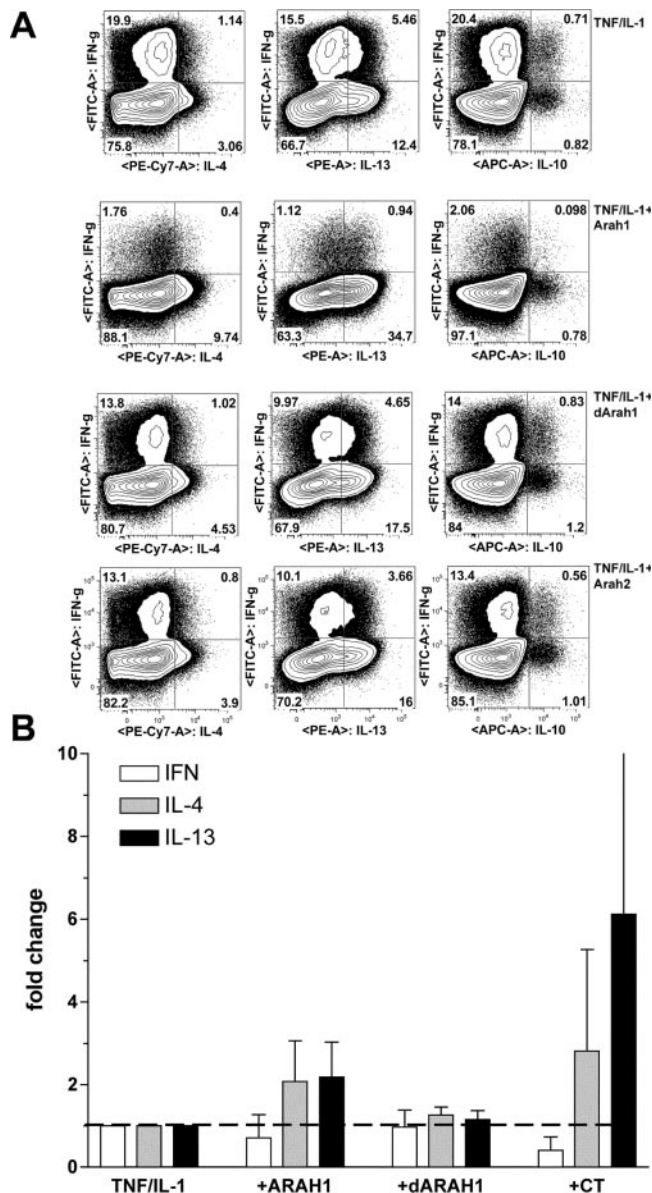


FIGURE 5. Ara h 1 is sufficient for activation and Th2 skewing of naive T cells. *A*, Example of intracellular cytokine data from CD3⁺CD4⁺ live T cells after 14-day coculture with Ara h 1-treated MDDCs showing increased IL-4- and IL-13-positive T cells compared with T cells cultured with MDDCs treated with TNF- α /IL-1 β . *B*, Summary of three independent experiments with different donors showing fold change in percentage of positive cells for IFN- γ , IL-4, or IL-13, as indicated. Error bars show SD.

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 α 1-3 fucose and β 1-2 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 α 1-3 fucose- and β 1-2 xylose-containing complex glycans derived from schistosome egg Ags are potent inducers of Th2 immune responses. Several defined α 1-3 fucose-bearing glycoconjugates have subsequently been identified as conferring at least some of this activity (13, 15, 42, 43).

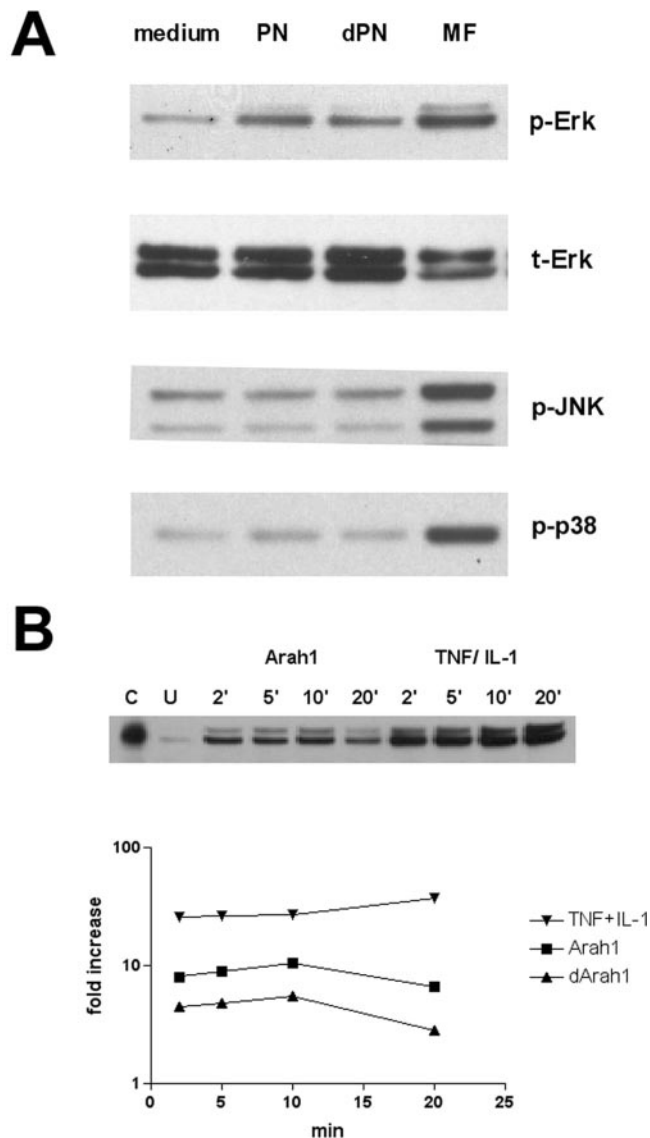


FIGURE 6. PNAg induces Erk 1/2 phosphorylation. *A*, Immunoblot of MDDC lysate for phosphorylated Erk 1/2, JNK, p38, and total Erk 1/2 after 5-min stimulation with PNAg, dPNAg, or TNF- α /IL-1 β . *B*, *Top*, Immunoblot of MDDC lysate for phosphorylated Erk 1/2 at indicated time points of stimulation with Ara h 1 or TNF- α /IL-1 β ; *bottom*, fold increase by densitometry in p-Erk/total Erk ratio over unstimulated cells for dAra h 1, Ara h 1, or TNF- α /IL-1 β . Data shown are representative of four independent experiments.

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 aqueous 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

mannan 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 naive 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 MDDCs 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 TLR-4 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 costimulatory 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/maturation 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-1 β 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.

Acknowledgments

We thank Adrian Ting and Lloyd Mayer for their generous advice both intellectual and technical.

Disclosures

The authors have no financial conflict of interest.

References

1. Aalberse, R. C., and S. O. Stapel. 2001. Structure of food allergens in relation to allergenicity. *Pediatr. Allergy Immunol.* 14(Suppl. 12): 10–14.
2. King, T. P., and M. D. Spangfort. 2000. Structure and biology of stinging insect venom allergens. *Int. Arch. Allergy Immunol.* 123: 99–106.
3. Pomes, A., and M. D. Chapman. 2001. Can knowledge of the molecular structure of allergens improve immunotherapy? *Curr. Opin. Allergy Clin. Immunol.* 1: 549–554.
4. Ghaemmaghami, A. M., A. Robins, L. Gough, H. F. Sewell, and F. Shakib. 2001. Human T cell subset commitment determined by the intrinsic property of antigen: the proteolytic activity of the major mite allergen Der p 1 conditions T cells to produce more IL-4 and less IFN- γ . *Eur. J. Immunol.* 31: 1211–1216.
5. Phillips, C., W. R. Coward, D. I. Pritchard, and C. R. Hewitt. 2003. Basophils express a type 2 cytokine profile on exposure to proteases from helminths and house dust mites. *J. Leukocyte Biol.* 73: 165–171.
6. Svensson, L., A. Rudin, and C. Wenneras. 2004. Allergen extracts directly mobilize and activate human eosinophils. *Eur. J. Immunol.* 34: 1744–1751.
7. Charbonnier, A. S., H. Hammad, P. Gosset, G. A. Stewart, S. Alkan, A. B. Tonnel, and J. Pestel. 2003. Der p 1-pulsed myeloid and plasmacytoid dendritic cells from house dust mite-sensitized allergic patients dysregulate the T cell response. *J. Leukocyte Biol.* 73: 91–99.
8. Chen, C. L., C. T. Lee, Y. C. Liu, J. Y. Wang, H. Y. Lei, and C. K. Yu. 2003. House dust mite *Dermatophagoides farinae* augments proinflammatory mediator productions and accessory function of alveolar macrophages: implications for allergic sensitization and inflammation. *J. Immunol.* 170: 528–536.
9. Traidl-Hoffmann, C., V. Mariani, H. Hochrein, K. Karg, H. Wagner, J. Ring, M. J. Mueller, T. Jakob, and H. Behrendt. 2005. Pollen-associated phytoprostanes inhibit dendritic cell interleukin-12 production and augment T helper type 2 cell polarization. *J. Exp. Med.* 201: 627–636.
10. Janeway, C., and R. Medzhitov. 2002. Innate immune recognition. *Annu. Rev. Immunol.* 20: 197–216.
11. Herre, J., A. S. Marshall, E. Caron, A. D. Edwards, D. L. Williams, E. Schweighoffer, V. Tybulewicz, E. S. C. Reis, S. Gordon, and G. D. Brown. 2004. Dectin-1 uses novel mechanisms for yeast phagocytosis in macrophages. *Blood* 104: 4038–4045.
12. Faveeuw, C., T. Malleval, K. Paschinger, I. B. Wilson, J. Fontaine, R. Mollicone, R. Oriol, F. Altmann, P. Lerouge, M. Capron, and F. Trottein. 2003. Schistosome *N*-glycans containing core α 3-fucose and core β 2-xylose epitopes are strong inducers of Th2 responses in mice. *Eur. J. Immunol.* 33: 1271–1281.
13. Thomas, P. G., and D. A. Harn, Jr. 2004. Immune biasing by helminth glycans. *Cell Microbiol.* 6: 13–22.
14. Okano, M., A. R. Satoskar, K. Nishizaki, M. Abe, and D. A. Harn, Jr. 1999. Induction of Th2 responses and IgE is largely due to carbohydrates functioning as adjuvants on *Schistosoma mansoni* egg antigens. *J. Immunol.* 163: 6712–6717.
15. Okano, M., A. R. Satoskar, K. Nishizaki, and D. A. Harn, Jr. 2001. Lacto-*N*-fucopentaose III found on *Schistosoma mansoni* egg antigens functions as adjuvant for proteins by inducing Th2-type response. *J. Immunol.* 167: 442–450.
16. Wilson, I. B., and F. Altmann. 1998. Structural analysis of *N*-glycans from allergenic grass, ragweed and tree pollens: core α 1,3-fucose and xylose present in all pollens examined. *Glycoconj. J.* 15: 1055–1070.
17. Wilson, I. B., R. Zeleny, D. Kolarich, E. Staudacher, C. J. Stroop, J. P. Kamerling, and F. Altmann. 2001. Analysis of Asn-linked glycans from vegetable foodstuffs: widespread occurrence of Lewis a, core α 1,3-linked fucose and xylose substitutions. *Glycobiology* 11: 261–274.

18. Kolarich, D., and F. Altmann. 2000. N-glycan analysis by matrix-assisted laser desorption/ionization mass spectrometry of electrophoretically separated non-mammalian proteins: application to peanut allergen Ara h 1 and olive pollen allergen Ole e 1. *Anal. Biochem.* 285: 64–75.
19. Okano, M., K. Nishizaki, A. R. Satoskar, T. Yoshino, Y. Masuda, and D. A. Harn, Jr. 1999. Involvement of carbohydrate on phospholipase A2, a bee-venom allergen, in vivo antigen-specific IgE synthesis in mice. *Allergy* 54: 811–818.
20. Dudler, T., F. Altmann, J. M. Carballido, and K. Blaser. 1995. Carbohydrate-dependent, HLA class II-restricted, human T cell response to the bee venom allergen phospholipase A2 in allergic patients. *Eur. J. Immunol.* 25: 538–542.
21. Faye, L., and M. J. Chrispeels. 1988. Common antigenic determinants in the glycoproteins of plants, molluscs and insects. *Glycoconj. J.* 5: 245–256.
22. Chrispeels, M. J. 1999. Glycobiology of plant cells. In *Essentials of Glycobiology*. A. Varki, R. D. Cummings, J. Esko, H. Freeze, G. Hart, and J. Marth, eds. Cold Spring Harbor Laboratory Press, Plainview.
23. Sicherer, S. H., A. Munoz-Furlong, and H. A. Sampson. 2003. Prevalence of peanut and tree nut allergy in the United States determined by means of a random digit dial telephone survey: a 5-year follow-up study. *J. Allergy Clin. Immunol.* 112: 1203–1207.
24. Burks, A. W., G. Cockrell, J. S. Stanley, and R. M. Helm. 1995. Recombinant peanut allergen Ara h I expression and IgE binding in patients with peanut hypersensitivity. *J. Clin. Invest.* 96: 1715–1721.
25. Tretter, V., F. Altmann, V. Kubelka, L. Marz, and W. M. Becker. 1993. Fucose α 1,3 linked to the core region of glycoprotein N-glycans creates an important epitope for IgE from honeybee venom allergic individuals. *Int. Arch. Allergy Immunol.* 102: 259–266.
26. Van Ree, R., M. Cabanes-Macheteau, J. Akkerdaas, J. P. Milazzo, C. Loutelier-Bourhis, C. Rayon, M. Villalba, S. Koppelman, R. Aalberse, R. Rodriguez, et al. 2000. β (1,2)-xylose and α (1,3)-fucose residues have a strong contribution in IgE binding to plant glycoallergens. *J. Biol. Chem.* 275: 11451–11458.
27. Wilson, I. B., J. E. Harthill, N. Mullin, D. Ashford, and F. Altmann. 1998. Core α 1,3-fucose is a key part of the epitope recognized by antibodies reacting against plant N-linked oligosaccharides. *Glycobiology* 8: 651–661.
28. Bardor, M., C. Faveeuw, A. C. Fitchette, D. Gilbert, L. Galas, F. Trottein, L. Faye, and P. Lerouge. 2003. Immunoreactivity in mammals of two typical plant glyco-epitopes, core α (1,3)-fucose and core xylose. *Glycobiology* 13: 427–434.
29. Figdor, C. G., Y. van Kooyk, and G. J. Adema. 2002. C-type lectin receptors on dendritic cells and Langerhans cells. *Nat. Rev. Immunol.* 2: 77–84.
30. Van Kooyk, Y., and T. B. Geijtenbeek. 2003. DC-SIGN: escape mechanism for pathogens. *Nat. Rev. Immunol.* 3: 697–709.
31. Van Die, I., S. J. van Vliet, A. K. Nyame, R. D. Cummings, C. M. Bank, B. Appelmek, T. B. Geijtenbeek, and Y. van Kooyk. 2003. The dendritic cell-specific C-type lectin DC-SIGN is a receptor for *Schistosoma mansoni* egg antigens and recognizes the glycan antigen Lewis^x. *Glycobiology* 13: 471–478.
32. Feinberg, H., D. A. Mitchell, K. Drickamer, and W. I. Weis. 2001. Structural basis for selective recognition of oligosaccharides by DC-SIGN and DC-SIGNR. *Science* 294: 2163–2166.
33. Geijtenbeek, T. B., G. C. van Duijnhoven, S. J. van Vliet, E. Krieger, G. Vriend, C. G. Figdor, and Y. van Kooyk. 2002. Identification of different binding sites in the dendritic cell-specific receptor DC-SIGN for intercellular adhesion molecule 3 and HIV-1. *J. Biol. Chem.* 277: 11314–11320.
34. Guo, Y., H. Feinberg, E. Conroy, D. A. Mitchell, R. Alvarez, O. Blixt, M. E. Taylor, W. I. Weis, and K. Drickamer. 2004. Structural basis for distinct ligand-binding and targeting properties of the receptors DC-SIGN and DC-SIGNR. *Nat. Struct. Mol. Biol.* 11: 591–598.
35. Agrawal, S., A. Agrawal, B. Dougherty, A. Gerwitz, J. Blenis, T. Van Dyke, and B. Pulendran. 2003. Cutting edge: different Toll-like receptor agonists instruct dendritic cells to induce distinct Th responses via differential modulation of extracellular signal-regulated kinase-mitogen-activated protein kinase and c-Fos. *J. Immunol.* 171: 4984–4989.
36. Dillon, S., S. Agrawal, K. Banerjee, J. Letterio, T. L. Denning, K. Oswald-Richter, D. J. Kasprovicz, K. Kellar, J. Pare, T. van Dyke, et al. 2006. Yeast zymosan, a stimulus for TLR2 and dectin-1, induces regulatory antigen-presenting cells and immunological tolerance. *J. Clin. Invest.* 116: 916–928.
37. Dillon, S., A. Agrawal, T. Van Dyke, G. Landreth, L. McCauley, A. Koh, C. Maliszewski, S. Akira, and B. Pulendran. 2004. A Toll-like receptor 2 ligand stimulates Th2 responses in vivo, via induction of extracellular signal-regulated kinase mitogen-activated protein kinase and c-Fos in dendritic cells. *J. Immunol.* 172: 4733–4743.
38. Burks, A. W., L. W. Williams, R. M. Helm, C. Connaughton, G. Cockrell, and T. O'Brien. 1991. Identification of a major peanut allergen, Ara h I, in patients with atopic dermatitis and positive peanut challenges. *J. Allergy Clin. Immunol.* 88: 172–179.
39. Khoo, K. H., H. H. Huang, and K. M. Lee. 2001. Characteristic structural features of schistosome cercarial N-glycans: expression of Lewis^x and core xylosylation. *Glycobiology* 11: 149–163.
40. Van Die, I., V. Gomord, F. N. Kooyman, T. K. van den Berg, R. D. Cummings, and L. Vervelde. 1999. Core α 1 $\rightarrow$ 3-fucose is a common modification of N-glycans in parasitic helminths and constitutes an important epitope for IgE from *Haemonchus contortus* infected sheep. *FEBS Lett.* 463: 189–193.
41. Nyame, A. K., A. M. Leppanen, B. J. Bogitsh, and R. D. Cummings. 2000. Antibody responses to the fucosylated LacdiNAc glycan antigen in *Schistosoma mansoni*-infected mice and expression of the glycan among schistosomes. *Exp. Parasitol.* 96: 202–212.
42. Van der Kleij, D., A. Van Remoortere, J. H. Schuitemaker, M. L. Kapsenberg, A. M. Deelder, A. G. Tielens, C. H. Hokke, and M. Yazdanbakhsh. 2002. Triggering of innate immune responses by schistosome egg glycolipids and their carbohydrate epitope GalNAc β 1-4(Fuc α 1-2Fuc α 1-3)GlcNAc. *J. Infect. Dis.* 185: 531–539.
43. Meyer, S., E. Van Liempt, A. Imbert, Y. Van Kooyk, H. Geyer, R. Geyer, and I. van Die. 2005. DC-SIGN mediates binding of dendritic cells to authentic pseudo-Lewis^x glycolipids of *Schistosoma mansoni* cercariae, the first parasite-specific ligand of DC-SIGN. *J. Biol. Chem.* 280: 37349–37359.
44. Appelmek, B. J., I. van Die, S. J. van Vliet, C. M. Vandenbroucke-Grauls, T. B. Geijtenbeek, and Y. van Kooyk. 2003. Cutting edge: carbohydrate profiling identifies new pathogens that interact with dendritic cell-specific ICAM-3-grabbing nonintegrin on dendritic cells. *J. Immunol.* 170: 1635–1639.
45. Cambi, A., K. Gijzen, J. M. de Vries, R. Torensma, B. Joosten, G. J. Adema, M. G. Netea, B. J. Kullberg, L. Romani, and C. G. Figdor. 2003. The C-type lectin DC-SIGN (CD209) is an antigen-uptake receptor for *Candida albicans* on dendritic cells. *Eur. J. Immunol.* 33: 532–538.
46. Caparros, E., D. Serrano, A. Puig-Kroger, L. Riols, F. Lasala, I. Martinez, F. Vidal-Vanaclocha, R. Delgado, J. L. Rodriguez-Fernandez, L. Rivas, et al. 2005. Role of the C-type lectins DC-SIGN and L-SIGN in *Leishmania* interaction with host phagocytes. *Immunobiology* 210: 185–193.
47. Geijtenbeek, T. B., S. J. Van Vliet, E. A. Koppel, M. Sanchez-Hernandez, C. M. Vandenbroucke-Grauls, B. Appelmek, and Y. Van Kooyk. 2003. Mycobacteria target DC-SIGN to suppress dendritic cell function. *J. Exp. Med.* 197: 7–17.
48. Tailleux, L., O. Schwartz, J. L. Herrmann, E. Pivert, M. Jackson, A. Amara, L. Legres, D. Dreher, L. P. Nicod, J. C. Gluckman, et al. 2003. DC-SIGN is the major *Mycobacterium tuberculosis* receptor on human dendritic cells. *J. Exp. Med.* 197: 121–127.
49. Rogers, N. C., E. C. Slack, A. D. Edwards, M. A. Nolte, O. Schulz, E. Schweighoffer, D. L. Williams, S. Gordon, V. L. Tybulewicz, G. D. Brown, and E. S. C. Reis. 2005. Syk-dependent cytokine induction by dectin-1 reveals a novel pattern recognition pathway for C type lectins. *Immunity* 22: 507–517.
50. Thomas, P. G., M. R. Carter, O. Atochina, A. A. Da'Dara, D. Piskorska, E. McGuire, and D. A. Harn. 2003. Maturation of dendritic cell 2 phenotype by a helminth glycan uses a Toll-like receptor 4-dependent mechanism. *J. Immunol.* 171: 5837–5841.
51. Brown, G. D., and S. Gordon. 2001. Immune recognition: a new receptor for β -glucans. *Nature* 413: 36–37.
52. Brown, G. D., J. Herre, D. L. Williams, J. A. Willment, A. S. Marshall, and S. Gordon. 2003. Dectin-1 mediates the biological effects of β -glucans. *J. Exp. Med.* 197: 1119–1124.
53. Gantner, B. N., R. M. Simmons, S. J. Canavera, S. Akira, and D. M. Underhill. 2003. Collaborative induction of inflammatory responses by dectin-1 and Toll-like receptor 2. *J. Exp. Med.* 197: 1107–1117.
54. Meyer-Wentrup, F., A. Cambi, G. J. Adema, and C. G. Figdor. 2005. "Sweet talk": closing in on C type lectin signaling. *Immunity* 22: 399–400.
55. Ito, T., Y. H. Wang, O. Duramad, T. Hori, G. J. Delespesse, N. Watanabe, F. X. Qin, Z. Yao, W. Cao, and Y. J. Liu. 2005. TSLP-activated dendritic cells induce an inflammatory T helper type 2 cell response through OX40 ligand. *J. Exp. Med.* 202: 1213–1223.
56. Amsen, D., J. M. Blander, G. R. Lee, K. Tanigaki, T. Honjo, and R. A. Flavell. 2004. Instruction of distinct CD4 T helper cell fates by different Notch ligands on antigen-presenting cells. *Cell* 117: 515–526.
57. Yvon, E. S., S. Vigouroux, R. F. Rousseau, E. Biagi, P. Amrolia, G. Dotti, H. J. Wagner, and M. K. Brenner. 2003. Overexpression of the Notch ligand, Jagged-1, induces alloantigen-specific human regulatory T cells. *Blood* 102: 3815–3821.
58. Sporri, R., and C. Reis e Sousa. 2005. Inflammatory mediators are insufficient for full dendritic cell activation and promote expansion of CD4⁺ T cell populations lacking helper function. *Nat. Immunol.* 6: 163–170.
59. Ekkens, M. J., Z. Liu, Q. Liu, J. Whitmire, S. Xiao, A. Foster, J. Pesce, J. VanNoy, A. H. Sharpe, J. F. Urban, and W. C. Gause. 2003. The role of OX40 ligand interactions in the development of the Th2 response to the gastrointestinal nematode parasite *Heligmosomoides polygyrus*. *J. Immunol.* 170: 384–393.
60. Maleki, S. J., R. A. Kopper, D. S. Shin, C. W. Park, C. M. Compadre, H. Sampson, A. W. Burks, and G. A. Bannon. 2000. Structure of the major peanut allergen Ara h I may protect IgE-binding epitopes from degradation. *J. Immunol.* 164: 5844–5849.