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Differential Polarization of Immune Responses by Plant 2S Seed Albumins, Ber e 1, and SFA8

Dorothy E. Kean,* Helen S. Goodridge,* Stephen McGuinness,* Margaret M. Harnett,* Marcos J. C. Alcocer,† and William Harnett2‡

The plant 2S seed albumins Ber e 1 and SFA8, although structurally very similar, vary with respect to their allergenic properties. Whereas the former represents a major allergen, the latter appears to promote only weak allergenic responses. The aim of this investigation was to determine whether the allergenic properties of Ber e 1 and SFA8 reflected differential polarization of dendritic cell (DC) and Th cell responses. We thus investigated the effect of recombinant forms of both allergens on DC and Th cell responses as indicated by cell surface phenotype and cytokine production. Exposure of murine DCs to SFA8, but not Ber e 1, resulted in production of the cytokines IL-12 p40 and TNF-α by a mechanism independent of recognition by TLRs. Furthermore, depending on the mouse strain used, increased expression of MHC class II and costimulatory molecules such as CD40, CD80, and CD86 was associated with exposure to SFA8, but not Ber e 1. In coculture experiments using the DO11.10 transgenic T cell that recognizes OVA peptide, DCs exposed to both allergens induced T cells to produce IFN-γ, but only Ber e 1 could induce significant production of IL-4 and IL-5. Likewise, analysis of transcription factors shows increased T-bet with respect to both allergens, but also GATA-3 with respect to Ber e 1. Overall, our data are consistent with the idea that the ability of Ber e 1, but not SFA8, to act as a potent allergen may reflect differences in their ability to induce IL-12 production. The Journal of Immunology, 2006, 177: 1561–1566.

Specific plant protein families are involved in many cases of allergy to food products. Toward this small and specific group of proteins, there appears to be a breakdown in tolerance that is manifested by an immune response that causes a direct hypersensitivity reaction. In this context, the cross-linking of mast cell-bound IgE Abs by specific proteins (allergens) represents the initial signal for the release of inflammatory mediators. Although several steps on this cascade of events are well described, the initial stages leading to the recognition of foreign proteins and the induction of an IgE Ab response, also called the sensitization phase, are less clear. Fundamental aspects as to whether a particular protein possesses some intrinsic characteristics responsible for inducing de novo IgE synthesis are still not defined. In a very few cases, it has been demonstrated that specific activity, for example proteolytic activity as is the case for Der p 1, the major house dust mite allergen, is essential to direct the adaptive immune response toward a Th2-dominated subset (1). However, only a limited number of allergens are proteases. Thus, similar effects arising from some unknown intrinsic property of Bet v 1 (pollen allergen) (2) and the house dust mite allergen Der f 2 have been observed (3).

To define which attributes of the plant proteins are essential for driving the polarization of the immune response, Ber e 1, the 2S albumin major allergen from Brazil nut seeds (Bertholletia excelsa), and SFA8, the 2S albumin from sunflower seeds (Helianthus annuus), a weak allergen, were used as model system. Historically, 2S albumins from seeds have been associated with allergy in the oil milling (castor and rapeseed) and baking industries, and several proteins from this group have been characterized as clinically important allergens (4–8). As a class, the 2S albumins are water-soluble proteins, products of multigene families, and are present in seeds of a wide range of dicotyledonous and monocotyledonous species (9) with an as yet unknown specific biological function. Structurally, the 2S albumins belong to the bifunctional inhibitor/lipid transfer protein/storage 2S albumin or plant prolamin superfamily (10) of proteins, characterized by their conserved antiparallel bundle of four α-helices held together by four disulfide bonds in a distinctive right-handed fold. A large number of known food allergens, such as plant α-amylase/trypsin inhibitors, 2S storage proteins, and lipid transfer proteins, belong to the prolamin superfamily and seem to have evolved from the same ancient gene (10).

Ber e 1 in particular is a plant 2S albumin allergen that gained notoriety when it was engineered into soybean to increase the nutritional value of the legume, and, hence, clinical data are readily available (8, 11). In contrast, the allergenicity of SFA8 has not been so convincingly demonstrated. Although there are reports of SFA8-binding IgE in the sera of some patients who are allergic to sunflower seeds (12–14), the overall incidence of sunflower seed allergy is very low, even in areas of high consumption such as Spain (pepas), Germany, and Greece. Interestingly, in the few reported cases, sunflower seed sensitization has been suggested to occur via inhalation rather than ingestion; hence, it correlates with the ownership of caged birds fed with sunflower seeds (15, 16) and with high pollen counts in the workplace (17). As consequence, sera from sunflower seed allergic patients generally cross-react with other protein families, such as Bet v 1 (17, 18), lipid transfer proteins, and higher m.w. fractions (19).

Many allergens, especially those derived from plant foods, are consistently more resistant to degradation by pepsin than other...
and key regulators of the immune response. In addition to pro-
MHC class II.

Brazil nut and sunflower 2S seed albumins

Materials and Methods

We examined the effects of these 2S albumins on the activation of dendritic cells (DCs), which are both sentinels and key regulators of the immune response. In addition to processing Ag and presenting it to naive CD4+ T cells, DCs can bias the subsequent immune response toward a Th1 or Th2 phenotype through costimulatory molecule interactions and the release of cytokines. IgE production is favored when B cells receive help from Th2 cells, and, hence, allergic responses are associated with a Th2 phenotype. We therefore examined the ability of recombinant forms of Ber e 1 and SFA8 to induce cytokine production and surface expression of costimulatory molecules by DCs, and thereby direct the DC-mediated polarization of subsequent Th cell responses. Our aim was thus to investigate the first steps in interaction between the allergens and the immune system in an attempt to obtain data that would help explain later immunological events such as priming of polarized effector T cell responses resulting in IgE Ab production. The model we used, coculture of DO11.10 transgenic T lymphocytes and DCs matured in the presence of the agent under analysis, is a standard assay when undertaking this form of study (26).

Materials and Methods

Brazil nut and sunflower 2S seed albumins

Recombinant Brazil nut and sunflower seed 2S albumins (Ber e 1 and SFA8) were produced and secreted full-length proteins by the yeast Pichia pastoris and purified by FPLC using a heparin-Sepharose column, as previously described (21). Both single SDS-PAGE band proteins (under denaturing conditions) were further purified as single peaks by preparative reverse-phase chromatography also as previously described (25). The reverse-phase purified recombinant proteins were shown to be absent of endotoxin, as determined by an Endosafe Kit (Charles River Laboratories). Our previous results have unequivocally established that the recombinant proteins mimic their native counterparts in structural and biophysical properties (21–23). Recent nuclear magnetic resonance results (21) and disulfide mapping by mass spectra (21) have shown that the four intrachain disulfide bridges were properly established during the protein expression, folding, and secretion in the yeast expression system used. The overall similarity in structure is such that we have recently managed to construct, express, and purify chimeric proteins (SFA8 × Ber e 1) by replacing common domains and used these chimeras to map the human IgE structural epitope of Ber e 1 (25). Furthermore, we have shown that both native and recombinant forms of Ber e 1 and SFA8 are equally stable to proteolytic digestion, an important attribute in the assessment of intrinsic allergenicity of proteins (22, 23). Finally, the immunoreactivity of the proteins by ELISA and radioallergosorbent test was examined using a panel of subjects allergic to the native nut protein, and in both cases it was found to mirror the native counterpart (22).

ELISA

IL-12 p40, TNF-α, IL-4, IL-5, and IL-10 ELISAs were performed according to the manufacturer’s instructions using paired Abs from BD PharMingen.

Flow cytometry

Flow cytometry was performed, as described previously (27). Cells were stained with Abs specific for CD11c in conjunction with Abs specific for MHC class II, CD40, CD54, B7.1 (CD80), B7.2 (CD86), along with the relevant isotype controls (BD PharMingen). Flow cytometry was conducted using a FACSCalibur Immunocytochemistry System (BD PharMingen).

DC culture

Wild-type mice (C57BL/6 and BALB/c) and OVA-specific TCR-transgenic DO11.10 mice (BALB/c) were from Harlan Olac; MyD88 knockout mice (C57BL/6 background) were a gift from S. Akira (University of Osaka, Osaka, Japan) and were maintained at the University of Manchester (Manchester, U.K.) with the assistance of K. Else. Femurs and tibias were dissected from mice and bone marrow (bm) cells seeded into 75-cm² flasks containing bm-DC culture medium (RPMI 1640 containing 2 mM glutamine, 50 U/ml penicillin, 50 µg/ml streptomycin, 10% FCS, 10% GM-CSF-transfected X63 myeloma cell line-conditioned medium, and 50 µM 2-ME) at 37°C in a humidified incubator (27). On day 4, the supernatant was carefully removed from the flasks, and the cells were replenished with fresh bm-DC culture medium. Two days later, loosely adherent cells were removed by gentle pipetting and centrifugation, and used as immature bm-DC. Following isolation, bm-DC were plated at 1 × 10⁶ cells/ml in 24-well plates and further grown in bm-DC culture medium in the presence or absence of stimulus (nut/seed allergen at 50 µg/ml) for a further 24 h at 37°C before analyzing DC maturation status (flow cytometric analysis of cell surface phenotype) and cytokine secretion (ELISA analysis of culture supernatants).

DC/T cell coculture

Day 6 bm-DC (isolated as described above) were plated at 1 × 10⁶ cells/ml in 24-well plates and grown in bm-DC culture medium in the presence or absence of stimulus (nut/seed allergen at 50 µg/ml) for a further 24 h at 37°C. The cells were then washed three times with PBS before coculturing with naive CD4+ DO11.10 transgenic T cells 1 day later. Purified naive CD4+ CD62Lhigh T cells from DO11.10/BALB/c mice were used as source of responder T cells for bm-DC derived from BALB/c mice. Spleens and lymph nodes were removed from DO11.10 mice and passed through Nitex. The cells were then washed in RPMI 1640 and resuspended (10⁵ cells/ml) in MACS buffer (FACSFlow; 0.5% FCS; BD Biosciences) and incubated with anti-CD4-MACS beads (Miltenyi Biotec). These purified CD4+ T cells were further purified to isolate (naive) CD62Llow T cells using anti-CD62L-MACS beads (Miltenyi Biotec). Purified naive (CD62LhighCD4+) transgenic T cells (10⁶ cells/well) were then cultured in RPMI 1640 plus 10% FCS with the relevant DC and various concentrations of endotoxin-free OVA peptide (0.03, 0.3, 3.0, and 30 nM) (323-ISQAVHAAHAEINEAGR-339; obtained from Genosys Europe) that would not automatically push polarization in a Th2 direction (26) for 3 days at 37°C. Three days later, cells were then pulsed overnight with [³H]thymidine (1 µCi/ml; Amersham) One day later, incorporation into DNA was analyzed following cell harvesting (Tomtec cell harvester; Wallac) by liquid scintillation counting (MicroBeta scintillation counter; Wallac). Alternatively, cells were stimulated with 50 ng/ml PMA (Sigma-Aldrich) and 500 ng/ml ionomycin (Sigma-Aldrich) overnight, and cell supernatants were then removed and tested for expression of IL-2, IL-4, IFN-γ, IL-10, and IL-12 p70 by ELISA, as indicated earlier.

TagMan real-time PCR

TagMan real-time PCR (28) was performed, according to the manufactur-er’s instructions (Applied Biosystems) and as described previously (29). The probes used were 5’FAM (reporter) and 3’TAMRA (quencher): GATA-3, forward primer 5’-3’-TCTCCTCTCCTACGGCTCCTTGCTA; reverse primer 3’-5’-CACTGATTTCTTTGGGCTGCT; probe 5’-3’-TCCTGATCGGGAAAGCAACCGCTC; Tbet, forward primer 5’-3’-GGCAGGAACCGCTTATAGT; reverse primer 3’-5’-AACTCTTGCGGCCACA TCCA; probe 5’-3’-CCCCAGACTTCCCCACACCGGA; and Foxp3, forward primer 5’-3’-GGAGGACCGACACACACCT; reverse primer 3’-5’-TCTCTACACACCCCGCCCTG; and probe 5’-3’-ATCCCTACACTGCTGCAATGAGGT. PCRs were performed in triplicate in an ABI PRISM 7700 sequence detector (Applied Biosystems). Data analysis
was performed using the Sequence Detection software (Applied Biosystems), and samples were normalized by their reference reporter hypoxanthine-guanine phosphoribosyltransferase.

**Statistics**

Statistical significance of ELISA data was calculated using Student’s t test.

**Results**

*Effects of 2S seed albumins on cytokine production by bm-DCs*

Treatment of DCs with pathogen molecules such as bacterial LPS results in strong induction of the Th1-biasing cytokine IL-12 as well as proinflammatory cytokines such as TNF-α. We therefore examined whether recombinant Ber e 1 and SFA8 are similarly able to induce such cytokine release by DCs. Treatment of bm-DCs from C57BL/6 mice with Ber e 1 did not result in the production of IL-12 or TNF-α (Fig. 1, A and B). In contrast, bm-DCs produced significant amounts of IL-12 p40 and TNF-α following exposure to SFA8 (Fig. 1, A and B). IL-12 and TNF-α induction was dose dependent, and a dose of 50 μg/ml was selected for additional experiments.

Neither of the two allergens interfered with the ability of the DC to respond to LPS (Fig. 1C). Such an ability has been noted with respect to pathogen products that interact with DCs via TLRs (e.g., the filarial nematode-derived phosphorylcholine-containing glycoprotein, ES-62) (30). However, induction of IL-12 by SFA8 does not appear to occur via interaction with a member of the TLR family of pattern recognition receptors, which are responsible for the innate recognition of a variety of pathogen (e.g., LPS) as well as host molecules, because IL-12 induction is intact in bm-DCs from C57BL/6 mice deficient in MyD88 (Fig. 1D). Similar to the C57BL/6 data, treatment of BALB/c bm-DCs with SFA8, but not LPS, is intact in bm-DCs from C57BL/6 mice deficient in MyD88 (Fig. 1, E and F, and results not shown).

bm-DCs from the Th2-biased BALB/c mouse strain responded to Ber e 1 and SFA8 in the same way as those from the C57BL/6 strain, which preferentially mounts Th1 responses. BALB/c bm-DCs produced IL-12 and TNF-α in response to treatment with SFA8, but not Ber e 1, which resulted in substantial up-regulation of MHC II, CD40, CD54, CD80, and CD86 (Fig. 3A). Indeed, apart from CD40 and CD80 expression that were more strongly induced by LPS (Fig. 3A) in comparison with the effects of SFA8 (Fig. 1A and B), the signaling adaptor required by most known TLRs for proinflammatory cytokine induction. Similarly, TNF-α production by SFA8, but not LPS, is intact in bm-DCs from C57BL/6 mice deficient in MyD88 (Fig. 1, E and F, and results not shown).

IL-10 levels in culture supernatants were also measured, but neither Ber e 1 nor SFA8 induced IL-10 production by DCs from either mouse strain at doses up to 50 μg/ml (data not shown).

**Effects of 2S seed albumins on costimulatory molecule expression by bm-DCs from C57BL/6 and BALB/c mice**

We next examined the effects of exposure to Ber e 1 and SFA8 on the expression of DC costimulatory molecules by flow cytometry. Consistent with widely published data, stimulation of bm-DCs from C57BL/6 mice with bacterial LPS induced a mature DC phenotype characterized by significant up-regulation of MHC class II (MHC II) as well as CD40, CD54, CD80 (B7.1), and CD86 (B7.2) surface expression (Fig. 2). In contrast, Ber e 1 did not significantly alter the expression of any of these molecules, indicating that C57BL/6 DCs exposed to Brazil nut albumins have an immature phenotype. SFA8 treatment induced marginal increases in MHC II, CD40, CD80, and CD86, but not CD54 expression (Fig. 2), indicating that while these albumins induce some degree of DC maturation, it is not to the same extent observed with LPS, a finding consistent with the low level of induction of IL-12 and TNF, resulting from stimulation of DCs with SFA8.

To investigate whether these effects on DC maturation are dependent on the mouse strain, we repeated the above experiment using bm-DCs derived from BALB/c mice. In contrast to the C57BL/6 data, treatment of BALB/c bm-DCs with SFA8, but not Ber e 1, resulted in substantial up-regulation of MHC II, CD40, CD54, CD80, and CD86 (Fig. 3A). Indeed, apart from CD40 and CD80 expression that were more strongly induced by LPS (Fig. 3B), SFA8 was almost as efficient as LPS in inducing a mature DC phenotype. Neither Ber e 1 nor SFA8, however, altered such LPS-induced DC maturation (Fig. 3B). Thus, while SFA8 is capable of inducing substantial DC maturation of BALB/c bm-DCs, Ber e 1 treatment results in DCs with a rather immature phenotype.
Effect of exposure of bm-DCs to 2S seed albumins on priming and polarization of naive Th cells

To investigate whether the differential DC maturation observed with Ber e 1 and SFA8 translates into differences in the Th response, we examined the ability of naive OVA-specific CD4+ T cells to respond to OVA presentation by bm-DCs pre-exposed to these 2S albumins. Others and we have shown previously that maturation of bm-DCs with LPS allows such OVA-pulsed DCs to prime naive OVA-specific CD4+ T cells (DO11.10) for proliferation and effector Th1 (high IFN-γ and low IL-4) polarization. We, therefore, investigated the effects of these 2S albumins relative to this well-established Th1 priming system. T cell proliferative responses to OVA presentation were found not to be significantly different between groups of DCs matured following pre-exposure of DCs to Ber e 1, SFA8, or LPS, although LPS-exposed DCs drove enhanced T cell proliferation at lower OVA concentrations than control DCs (Fig. 4A). Cytokine induction following DC/T cell coculture was also assessed. Interestingly, and rather to our surprise given their apparent immature phenotype, DCs pre-exposed to Ber e 1 induced significantly more IFN-γ than those matured with LPS (Fig. 4B). DCs treated with SFA8 also tended to induce higher levels of IFN-γ than those exposed to LPS, but this effect was not statistically significant. However, Ber e 1-treated DCs primed Th cells to produce significantly more IL-4 than those matured with LPS (Fig. 4C). Similarly, Ber e 1-matured DCs induced substantially higher levels of IL-5 and, to a lesser extent, elevated levels of IL-10, than those cultured with LPS (Fig. 4, D and E). Moreover, levels of the Th2 cytokines IL-4 and IL-5 were lower in cultures containing SFA8-exposed DCs than those with Ber e 1-exposed DCs, and indeed were almost comparable to those obtained with LPS-matured DCs.

To further characterize the phenotype of the Ag-specific effector Th cells resulting from exposure to 2S albumins, the profile of expression of transcription factors associated with Th1 (T-bet), Th2 (GATA-3), and T regulatory/IL-10-producing Th cell (Foxp3) responses was determined in such OVA-specific DC/T cell cultures. Consistent with the high levels of IFN-γ release observed, all groups displayed elevated T-bet mRNA levels (Fig. 4F) with Ber e 1-treated cultures showing the highest levels and the LPS and SFA8 cultures showing the same rank order as for IFN-γ. Similarly, and consistent with the elevated IL-4 and IL-5 observed, GATA-3 levels were highest in T cells cultured with Ber e 1-treated DCs (Fig. 4G). By contrast, there was no apparent induction of Foxp3 in any of the cultures (Fig. 4H). Analysis of DCs from culture in the absence of T cells showed no induction of T-bet, GATA-3, or Foxp3 (results not shown).

Discussion

In this study, we investigated the ability of 2S seed albumins to polarize the immune response via modulation of DC activation. SFA8, which is not considered to be strongly allergenic, induced the production of Th1-promoting cytokines by DCs (BALB/c and C57BL/6 mouse strains), and drove the maturation of BALB/c DCs with the capacity to induce T cell proliferation, high IFN-γ production, and little or no IL-4 or IL-5 production. In contrast, Ber e 1, which is a well-established allergen, did not induce IL-12 or TNF-α production by DCs or the phenotypic maturation of DCs derived from either the BALB/c or the C57BL/6 mouse strains. Furthermore, although Ber e 1-exposed DCs did not differ from LPS- or SFA8-matured DCs in their ability to drive T cell proliferation or produce IFN-γ, they were also able to induce the production of the Th2 cytokines IL-4, IL-5, and IL-10. The surprising finding that Ber e 1-exposed DCs produced the highest levels of IFN-γ may reflect the recent report that while IL-4 initiates Th2
from atopic patients produced less IL-12 in response to CD40 ligand failure of DCs to produce IL-12 and atopy. PBMC-derived DCs system. Furthermore, the data presented in this study are consistent with the Th1-type polarization observed when lupin-containing transgenic SFA8 was administered orally to animal models of experimental asthma (32). Rather, it acts to promote a Th2 environment and thus the development of an allergic response. In contrast, SFA8, which is considered to be a weak allergen, is capable of inducing IL-12 production that may be important in determining initiation of allergic responses to proteins that are incapable of inducing IL-12 production by DCs.

Interestingly, costimulatory molecule expression was induced more dramatically by SFA8 in DCs from BALB/c mice than those from C57BL/6 mice. This may be due to strain-specific differences in the Th bias of these mouse strains. Hence, SFA8 exposure may have a more dramatic Th1-biasing effect on BALB/c DCs, which preferentially mount Th2 responses, than it does on C57BL/6 DCs, which are already Th1 biased. The mechanism of differential recognition of 2S seed albumins by DCs is unclear. The ability of SFA8, but not Ber e 1, to trigger proinflammatory cytokine production by DCs may be a reflection of the nature of its binding to DC surface receptors. However, our data indicate that SFA8 is not recognized by a member of the TLR family of pattern recognition receptors because the induction of IL-12 or TNF-α was not altered in the absence of MyD88, a key signaling adaptor used by most of the known TLRs. Moreover, these results confirm that the response observed with SFA8 is not due to endotoxin contamination occurring during the purification procedure.

A recent study demonstrated strain-specific differences in a mouse allergy model. Oral challenge with cow’s milk or peanut proteins following oral sensitzation with these proteins in the presence of cholera toxin resulted in anaphylaxis and dramatically elevated levels of allergen-specific IgE levels and serum histamine in C3H/HeJ, but not BALB/c mice (35). Furthermore, splenocytes from these C3H/HeJ mice responded to ex vivo culture in the presence of the relevant Ag by producing the Th2 cytokines IL-4 and IL-10, whereas the BALB/c splenocytes produced the Th1 cytokine IFN-γ. However, our present results may now suggest that with respect to potential allergens, high production of IFN-γ may not simply reflect a polarization to a Th1 phenotype, as in our in vitro DC-T cell cocultures, Ber e 1-matured DCs primed Th cells for production of IL-4, IL-5, and high levels of IFN-γ. Thus, as by contrast, LPS- and SFA8-matured DCs only primed for IFN-γ responses, it is possible that it is the ratio of IL-4/5 to IFN-γ production that may be important in determining initiation of allergy.

The intrinsic molecular property that promotes the observed differences between the two proteins remains unclear. The two proteins studied in this work were highly purified, properly folded recombinant products from single genes that similarly to their native counterparts are composed of a mixture of internal posttranslational processing units, albeit in different ratios. Thus, native plant 2S albumins are heterodimeric proteins, products of multigene families, synthesized as precursor proteins that after folding are sorted into vesicles for further transport to the vacuole (21). Most 2S plant albumins are heterodimeric proteins, products of multigene families, synthesized as precursor proteins that after folding are sorted into vesicles for further transport to the vacuole (21). Most 2S plant albumins are heterodimeric proteins, products of multigene families, synthesized as precursor proteins that after folding are sorted into vesicles for further transport to the vacuole (21). Most 2S plant albumins are heterodimeric proteins, products of multigene families, synthesized as precursor proteins that after folding are sorted into vesicles for further transport to the vacuole (21). Most 2S plant albumins are heterodimeric proteins, products of multigene families, synthesized as precursor proteins that after folding are sorted into vesicles for further transport to the vacuole (21). Most 2S plant albumins are heterodimeric proteins, products of multigene families, synthesized as precursor proteins that after folding are sorted into vesicles for further transport to the vacuole (21). Most 2S plant albumins are heterodimeric proteins, products of multigene families, synthesized as precursor proteins that after folding are sorted into vesicles for further transport to the vacuole (21). Most 2S plant albumins are heterodimeric proteins, products of multigene families, synthesized as precursor proteins that after folding are sorted into vesicles for further transport to the vacuole (21). Most 2S plant albumins are heterodimeric proteins, products of multigene families, synthesized as precursor proteins that after folding are sorted into vesicles for further transport to the vacuole (21). Most 2S plant albumins are heterodimeric proteins, products of multigene families, synthesized as precursor proteins that after folding are sorted into vesicles for further transport to the vacuole (21). Most 2S plant albumins are heterodimeric proteins, products of multigene families, synthesized as precursor proteins that after folding are sorted into vesicles for further transport to the vacuole (21). Most 2S plant albumins are heterodimeric proteins, products of multigene families, synthesized as precursor proteins that after folding are sorted into vesicles for further transport to the vacuole (21). Most 2S plant albumins are heterodimeric proteins, products of multigene families, synthesized as precursor proteins that after folding are sorted into vesicles for further transport to the vacuole (21). Most 2S plant albumins are heterodimeric proteins, products of multigene families, synthesized as precursor proteins that after folding are sorted into vesicle...
With the recent availability of the chimeric proteins (SFA8 × Ber e 1) (25), we hope soon to be able to address each of these points with respect to the two proteins. It is certainly of interest that it has been shown recently that microbial products that have different polarizing properties are acquired by DCs via discrete pathways and enter nonoverlapping intracellular compartments (36), and it has been suggested that such behavior represents a significant component of the ability of DC to polarize subsequent immune responses. Thus, one explanation for the differences that we observe with the two seed albumins may be that they mirror the microbial products with respect to differences in DC entry and compartmentalization. Again, this is something we plan to explore.

Thus, to summarize, although Ber e 1 and SFA8 are very similar with respect to size and structure, they have very different effects on DC function. Only the latter molecule can induce costimulatory interaction. These differences can explain the observed dissimilarity in allergenicity of the two molecules, but the intrinsic feature that promotes the differences remains to be elucidated.

Disclosures
The authors have no financial conflict of interest.

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