

Differences in the Uptake of Ara h 3 from Raw and Roasted Peanut by Monocyte-Derived Dendritic Cells

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Keywords

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Abstract

Roasting has been implicated in the increase of peanut allergenicity due to the chemical reactions that occur during the process. However, this increase is not fully understood, and little information is available regarding the role of roasted peanut allergens in the initial phase of allergy, where dendritic cells (DCs) play a key role. We sought to analyze differences in the internalization of Ara h 3 from raw and roasted peanut by immature monocyte-derived DCs (MDDCs) and the implication of the mannose receptor in the uptake. Ara h 3 was purified from raw and roasted peanut (Ara h 3-raw and Ara h 3-roas) and labeled with a fluorescent dye. The labeled allergens were added to MDDCs obtained from 7 donors and internalization was analyzed after 10, 30, and 120 min by flow cytometry. In parallel, mannan, which blocks the mannose receptor, was added 30 min before adding the labeled allergens. Results showed that the internalization of Ara h 3-roas by MDDCs was significantly increased at every time point. However, the increase in the internalization

of Ara h 3-raw was only significant after 2 h of incubation. Ara h 3-roas had an enhanced capacity to be internalized by MDDCs in comparison with Ara h 3-raw at every time point. Blocking the mannose receptor decreased the internalization of Ara h 3-roas but not Ara h 3-raw. In conclusion, the internalization of Ara h 3-roas by the MDDCs is enhanced when compared to Ara h 3-raw, and the mannose receptor might be implicated in this enhancement.

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Peanut allergy represents one of the most severe food allergies due to its persistence and life-threatening nature [1]. Up to now, 16 allergens from peanut have been described and officially registered [2]. Peanuts are frequently subjected to thermal processing prior to consumption, and the method used for this can impact on the allergenicity of peanut. Allergens from roasted peanut have a higher capacity to bind peanut-specific IgE from the sera of peanut-allergic patients than raw, boiled, or fried peanut [3–5]. The chemical reactions that occur during

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roasting seem to play a pivotal role in the increased capacity of peanut allergens to bind peanut-specific IgE in the elicitation phase of the allergic response [3, 6, 7]. However, little information is available regarding the role of roasted peanut allergens in the initial phase of allergy, the so-called “sensitization” phase, where an immunological priming towards an allergic response takes place. At the initial stage of sensitization, DCs, with several mechanisms and pathways for antigen uptake, are specialized for antigen capture, processing, presentation to T cells, and the priming of these cells into CD4⁺ Th1, Th2, Th0, tolerogenic T-cell subtypes (among others) to initiate an adaptive immune response. DCs are thus thought to play a key role in the induction of immune responses such as allergy or tolerance [8, 9]. Some previous studies have found that glycosylated allergens can be recognized by DCs through specific type of receptors [10], increasing the allergen uptake by the DCs [11–14]. DCs use several mechanisms for antigen uptake such as receptor-mediated endocytosis, micropinocytosis, or phagocytosis. The different antigen uptake mechanisms and receptors involved may depend on the characteristics of the specific antigens. For many allergens, the uptake mechanisms and their consequences still need to be elucidated.

We hypothesized that the roasting-related changes in the chemistry of peanut allergens would increase the potential of those allergens to be recognized by specific receptors expressed by DCs, and favor an enhanced allergen uptake. In this study, we investigated differences in the internalization of Ara h 3 purified from raw and roasted peanut by MDDCs and the implication of the mannose receptor in the uptake.

Ara h 3 was purified from raw or roasted peanut by ammonium sulfate precipitation followed by ion-exchange chromatography. Raw peanut flour was defatted for 6 h using petroleum ether. Roasted peanut flour was a generous gift from Golden Peanut Co. (Modesto, CA, USA). The defatted flour (10 g) was solubilized in 500 mL of extraction buffer (50 mM Tris-Cl, pH 8.3, 10 mM DTT, 5 mM EDTA, and 1 mM PMSF) containing 200 mM NaCl. The solution was homogenized with ultrasound treatment on ice at 40% power using a heat systems disrupter. The homogenate was cleared by centrifugation at 13,000 rpm for 30 min at 4°C. Ara h 3 was precipitated from the cleared homogenate by addition of a saturated solution of ammonium sulfate while stirring, and stirring was continued for 30 min on ice. The ammonium sulfate precipitated proteins were collected by centrifugation at 13,000 rpm for 30 min at 4°C and stored stably at 4°C. The ammonium sulfate pellet was solubilized in extraction buffer

(50 mM Tris, and 1 mM EDTA; pH 7). Solubilized proteins were then subjected to ion-exchange chromatography and eluted with a salt gradient. Purified Ara h 3 from raw (Ara h 3-raw) or roasted (Ara h 3-roas) peanut was labeled with the DyLight amine-reactive dye 488 NHS ester system (Thermo Fisher Scientific, Bremen, Germany) according to the manufacturer’s instructions. Briefly, labeling solution containing NHS-ester dye was added to Ara h 3-raw and Ara h 3-roas solution (2.5 mg/mL), and then incubated at room temperature for 1 h in the dark. Nonreacted dye was removed by dialyzing for 4 h. The labeled proteins were stored at –20°C and protected from light until use.

After obtaining informed consent, peripheral blood samples were drawn from 7 healthy, nonallergic donors. The study was approved by the Ethics Committee of the University of Bonn. Monocytes were isolated from peripheral blood with a modified density gradient protocol using Nycoprep (Progen Biotechnik GmbH, Heidelberg, Germany) [15]. They were cultured for 6 days in VLE-RPMI 1640 (Biochrom AG, Berlin, Germany) with 1% antibiotics/antimycotics and 10% inactivated FCS, 500 U/mL GM-CSF (Berlex Laboratories, Richmond, CA, USA), and 500 U/mL IL-4 (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany). They were fed every second day with half of the cytokine dosage to generate MDDCs at immature stage. CD1a expression was used as a marker of immature MDDCs, measured by flow cytometry, using an anti-human CD1a APC-conjugated antibody and the corresponding isotype control (BD Biosciences, Heidelberg, Germany).

MDDCs were collected by gentle pipetting, washed, and resuspended in RPMI 1640 with 1% antibiotics/antimycotics and 10% inactivated FCS. They were then plated in 96-well, tissue-culture, flat-bottom plates (Corning Inc., NY, USA) at 5×10^4 cells/well, and incubated at 37°C in the same medium with labeled Ara h 3-raw or labeled Ara h 3-roas at 50 µg/mL. They were collected after 10, 30, and 120 min of allergen incubation. The end-time point of 120 min was selected based on previous studies [11, 16]. MDDCs were washed, and fixed with formaldehyde 4% in PBS during 20 min at RT. Cells were washed and allergen internalization was measured by FACSCanto flow cytometer (BD Biosciences). Uptake was measured as mean fluorescence intensity (MFI). In addition, mannan, a mannose receptor blocker [11, 12, 17] (Sigma-Aldrich, Taufkirchen, Germany), was added at 20 µg/mL to the MDDCs 30 min before stimulation with labeled Ara h 3-raw/-roas, and it was present during this step of allergen stimulation.

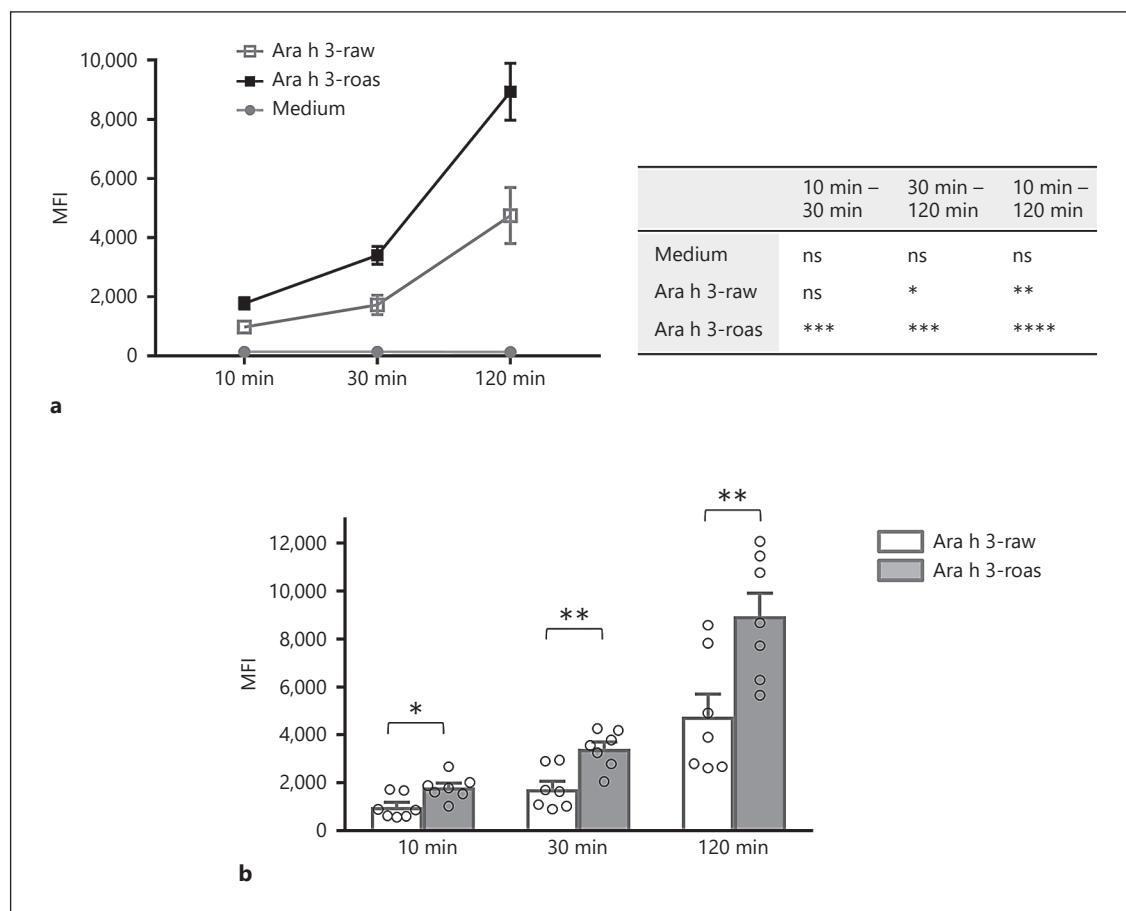


Fig. 1. a Internalization of Ara h 3-raw and Ara h 3-roas by MD-DCs at different time points. MDDCs were incubated with labeled Ara h 3-raw, Ara h 3-roas, or medium alone for 10, 30, and, 120 min. Allergen internalization was measured by flow cytometry and expressed as mean fluorescence intensity (MFI); $n = 7$ donors. Results are expressed as mean $\pm$ SEM. The annexed table shows the statistical results for each time interval. **b** Comparison in the same

experiment of the internalization of Ara h 3-raw (white bars) and Ara h 3-roas (grey bars) by MDDCs at 10, 30, and 120 min of allergen incubation. Allergen internalization was measured by flow cytometry and expressed as MFI; $n = 7$ donors. Results are expressed as mean $\pm$ SEM. Individual values are also shown. ns, not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

The results obtained from the different donors are expressed as mean $\pm$ SEM. Student's t test was used to analyze the statistical significances. Differences were considered significant with $p < 0.05$. The statistics software GraphPad Prism v7 for Windows (GraphPad Software, San Diego, CA, USA) was used.

We analyzed allergen internalization of Ara h 3 from raw and roasted peanut by immature MDDCs. Online supplementary Figure 1 (see www.karger.com/doi/10.1159/000489277) shows the purified Ara h 3-raw and Ara h 3-roas used in this study. The high expression of CD1a by immature MDDCs is also depicted. Figure 1a shows that fluorescence intensity progressively increased from 10 to 120 min of allergen incuba-

tion, demonstrating that Ara h 3 uptake by MDDCs was time-dependent. Interestingly, the increase in the internalization of Ara h 3-roas was significant at every time point, with a robust significant increase at 120 min ($p < 0.0001$); in contrast, the increase in the internalization of Ara h 3-raw was only significant after 2 h of allergen incubation ($p < 0.01$) (Fig. 1a, annexed table).

In the same experiment, we compared Ara h 3-raw and Ara h 3-roas internalization by MDDCs. Figure 1b shows that the internalization of Ara h 3-roas by MDDCs was significantly enhanced in comparison with Ara h 3-raw after 10 ($p < 0.05$), 30 ($p < 0.01$), and 120 min ($p < 0.01$) of allergen incubation.

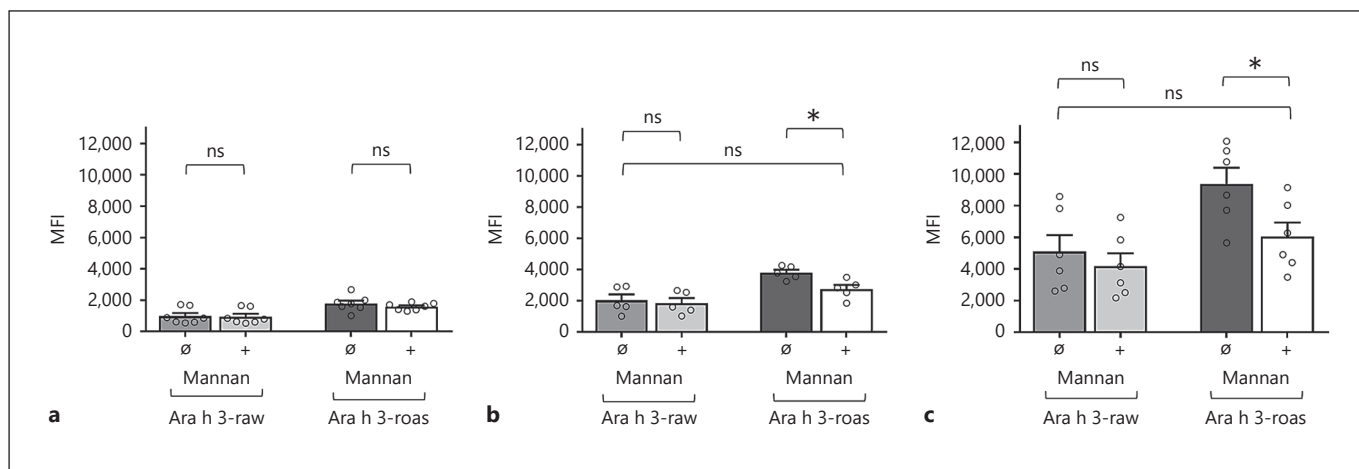


Fig. 2. Role of mannose receptor in the uptake and internalization of Ara h 3-raw and Ara h 3-roas by MDDCs. Mannan was added (+) to MDDCs 30 min before Ara h 3-raw or Ara h 3-roas incubation, internalization of the allergens was measured after 10 min ($n = 7$ donors) (**a**), 30 min ($n = 5$ donors) (**b**), and 120 min ($n = 6$

donors) (**c**), and compared with internalization in a mannann-free medium ($\emptyset$). Allergen internalization was measured by flow cytometry and expressed as MFI. Results are expressed as mean $\pm$ SEM. Individual values are also shown. ns, not significant; * $p < 0.05$.

We were next interested in the role of the mannose receptor in the uptake and internalization of Ara h 3-raw and Ara h 3-roas by MDDCs. Therefore, we added mannann to MDDCs as a blocker of the mannose receptor [11, 12, 17], 30 min before incubation with the labeled allergens. The internalization of the allergens was measured at 10, 30, and 120 min, and compared with internalization in a mannann-free medium. The results showed that mannann was able to significantly decrease the internalization of Ara h 3-roas after 30 ($p < 0.05$) and 120 min ($p < 0.05$) of allergen incubation (Fig. 2b, c). The addition of mannann did not significantly decrease the uptake of Ara h 3-raw at any time point (Fig. 2). Interestingly, blocking of the mannose receptor by mannann decreased the internalization of Ara h 3-roas up to levels of fluorescence intensity similar to Ara h 3-raw (Fig. 2b, c), which suggests that the mannose receptor might be responsible for the increased internalization observed for Ara h 3-roas.

It is commonly agreed that allergens from roasted peanut have an increased capacity to bind preformed peanut-specific IgE compared to raw peanut, as this has been demonstrated for several peanut allergens, including the Ara h 3 samples of this study [3, 6, 7, 18]. However, the interaction of such thermal-processed peanut allergens with DCs in the sensitization phase of allergy is poorly understood. In this study, we focused on Ara h 3 purified from raw and roasted peanut, and showed that Ara h

3-roas had an enhanced capacity to be taken up and internalized by immature MDDCs in comparison with Ara h 3-raw. This result indicates that increased and/or different receptors could be involved in the enhanced uptake and internalization of Ara h 3-roas by MDDCs. During roasting, chemical reactions occur, which can modify peanut allergens and their interaction with the immune system [6, 7, 9]. One of the reactions that may favor this mechanism is the Maillard reaction, which is a nonenzymatic chemical reaction that occurs during roasting that implies modifications of amino groups of proteins by reducing sugars, thereby generating glycosylated proteins called advanced glycation end (AGE) adducts [3, 19]. The high temperatures reached during roasting (around 170 °C) would facilitate this reaction.

The chemical modifications taking place during roasting would therefore alter Ara h 3 chemistry in a way that is recognized by more/different receptors than the same allergen from raw peanut. We were able to confirm this hypothesis, demonstrating that the mannose receptor is involved in the enhanced capacity of MDDCs to take up and internalize Ara h 3-roas. The mannose receptor, a member of the C-type lectin receptor family, has been implicated in the recognition of glycosylated allergens through its carbohydrate-recognition domains [11, 12, 17]. This supports the hypothesis that the mannose receptor might be responsible for the increased recognition of Ara h 3-roas in this study. Other members of the C-

type lectin receptor family have been implicated in the recognition of other peanut allergens, such as Ara h 1, by MDDCs playing a direct role in priming a Th2 response [14].

In conclusion, our study demonstrated that, even in healthy donors, the uptake and internalization of Ara h 3-roas by MDDCs is enhanced when compared to Ara h 3-raw, and the mannose receptor might be implicated in this enhancement. Our study focused on Ara h 3, since it has been described as a major allergen in specific allergic study populations [2, 20, 21]. However, further studies would be necessary to analyze if similar results can be achieved for other allergens from peanut.

The results of this study may stimulate further research into the role of roasted peanut allergens in the immunological priming toward an allergic response in the sensitization phase of allergy.

Disclosure Statement

The authors have no conflicts of interest to declare.

Funding Sources

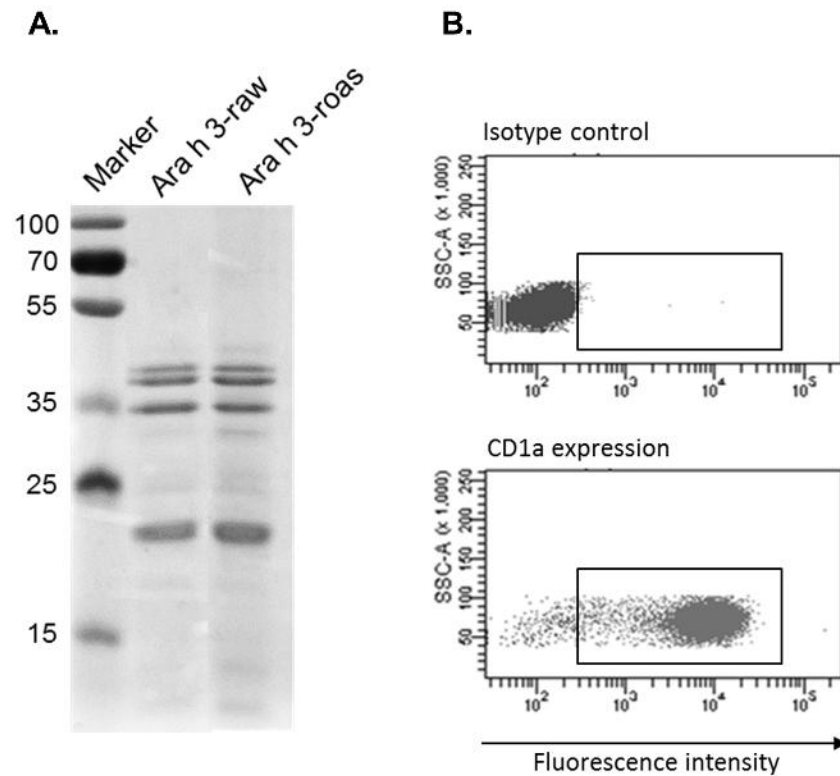
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Author Contributions

Study concept and design: B.C., N.N.; purification of Ara h 3: H.C., S.J.M.; protein labeling and MDDC experiments: B.C.; and data analysis: B.C., N.N. All authors contributed to the drafting of the manuscript and/or to its critical revision for important intellectual content.

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Supplementary material. Figure 1. A. SDS-PAGE (12%) of purified Ara h 3-raw and Ara h 3-roas (3 μ g protein / well). B. CD1a expression in immature MDDCs measured by flow cytometry. The picture shows a representative experiment.