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Dry roasting enhances peanut allergic sensitization across mucosal and cutaneous routes in mice

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To the Editor

The reasons for the disproportionate contribution of peanuts to prevalent and severe cases of food allergy in the Western world are unclear. Emerging statistics from East Asia generally match the overall common food allergies in the West, with the striking exception of peanuts, which are consumed equivalently in both regions.^{1, 2} Differences in peanut preparation, roasted and dry-roasted (DR) in the West versus raw, boiled, or fried in the East, have been proposed to contribute to this trend,^{1–3} and are supported by serological studies.^{1, 4} Results obtained with other proteins have highlighted the potential immunomodulatory properties of advanced glycation end products (AGE),⁵ which are extensively formed during the high temperature dry roasting of peanuts.⁶ However, despite this information, the immunogenicity and allergenicity of raw compared to DR peanuts has not been characterized in vivo.

To address this, we first primed BALB/c mice subcutaneously (SC) with endotoxin-depleted soluble fractions of peanut protein extract (PPE) from raw or DR peanuts in PBS without adjuvant (see Fig E1, A, E2, and E3, A, and Methods in this article's Online Repository at www.jacionline.org). This resulted in enhanced peanut-specific IgG titers in DR-primed groups across a range of doses, reactive against both raw and DR peanut extracts, and with a dominant IgG1:IgG2a bias (see Fig E4, A–C, in this article's Online Repository at www.jacionline.org). After three subsequent intra-gastric (IG) gavages of endotoxin-undetectable DR crude peanut homogenate (CPH; see Fig E2, and E3, A), anti-raw peanut

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IgG titres rose by an average of 100-fold more in the DR compared to the raw PPE group, and was associated with significant titers of anti-raw IgE, functional in basophil degranulation (Fig 1, *A* and *B*). Mesenteric lymph node (MLN) cells from DR but not raw PPE-primed mice proliferated robustly in response to raw and DR PPE, and cytokines produced were dominated by IL-4 and IL-5 but not IFN- γ and TNF- α (Fig 1, *C* and *D*, and see Fig E4, *D* and *E*). This pattern of enhanced DR PPE immunogenicity was maintained in a similar experiment, where PPE and CPH were kept homologous (raw/raw or DR/DR) during the SC prime and IG exposures (see Fig E4, *F*). In a further experiment we fed mice previously primed with DR or raw PPE with raw peanut kernels (see Fig E3, *B*) and observed significantly increased anti-raw and anti-DR PPE IgE only in DR-primed mice (Fig 1, *E*, and see E4, *G*). Finally, we excluded SC priming and directly administered multiple adjuvant-free IG CPH gavages (see Fig E3, *C*, and Repository Methods). DR but not raw CPH elicited anti-peanut IgG1 and functional IgE responses (Fig 1, *F–H*, and see Fig E5 in this article's Online Repository at www.jacionline.org).

Atopic dermal sensitization could be highly relevant to peanut allergy.⁷ We therefore adopted an epicutaneous (EC) sensitizing strategy described previously (see Fig E3, *D*, and Repository Methods), with raw or DR PPE applied daily to a developing atopic dermatitis-like skin lesion induced by topical administration of the exfoliating vitamin D analogue calcipotriol (MC903). Subsequent IG gavage and whole peanut feeding elicited higher peanut-specific IgG and IgE titers in the DR compared to raw PPE-sensitized mice (Fig 1, *I* and *J*, and see Fig E6 in this article's Online Repository at www.jacionline.org). Moreover, DR PPE-sensitized mice showed higher eosinophilic infiltration of lamina propria (LP) and increased IL-4, IL-5 and IL-13 secretion by MLN cells (Fig 1, *K–M*). Taken together, these data consolidate an enhanced Th2/allergenic profile of DR compared to raw peanuts, maintained across SC, EC and GI routes of sensitization.

Dry roasting modifies the physicochemical properties of peanut proteins via the Maillard reaction, in a manner that may influence protein immunogenicity via cross-linking and/or AGE adduction.^{4–6} We excluded cross-linking as a major contributor to immunogenicity since our DR PPE samples underwent multiple rounds of filtration and centrifugation that depleted cross-linked species (see Fig E1). Furthermore, we purified major peanut allergen Ara h1 from PPE and observed by native gel electrophoresis and multi-angle light scattering that compared to raw, DR peanut-derived Ara h1 retained fewer multimers (see Fig E1, *C* and *D*), yet was more immunogenic in a SC immunization of BALB/c mice (see Fig E3, *E* and E4, *H*). However, consistent with the presence of AGE-related adducts,⁶ DR but not raw Ara h1 bound to two well-characterized AGE receptors CD36 and RAGE,^{8,9} and DR PPE binding to bone marrow-derived dendritic cells (BMDCs) was partially inhibited by scavenger receptor blockers (Fig 2, *A* and *B*). Consistent with this, DR PPE was more efficient than raw PPE in inhibiting the in vitro presentation of maleylated or AGE-modified hen egg lysozyme (HEL) by BMDCs to a HEL-specific T cell hybridoma (Fig 2, *C*, and see Online Repository Methods).

These results confirm the presence of biologically active AGE adducts in DR peanut proteins, and their ability to target these proteins to antigen presenting cells. Phenotypic and cytokine profile analyses of peanut-pulsed BMDCs, however, revealed no classical

activation by raw or DR peanut preparations as compared to lipopolysaccharide (LPS) (Fig 2, *D* and *E*, and see Fig E7, *A–C*, in this article's Online Repository at www.jacionline.org). Moreover, by contrast with direct AGE-relevant modification of HEL that showed increased presentation to an HEL-specific T cell hybridoma, DR PPE-pulsed DCs failed to enhance presentation of unmodified HEL, reinforcing the absence of DR PPE-elicited DC costimulation (Fig 2, *F*, and see Fig E8 in this article's Online Repository at www.jacionline.org). Finally, we confirmed the lack of canonical pro-inflammatory activation by raw or DR PPE and CPH preparations using sensitive NF κ B/AP1 murine and human monocyte/macrophage reporter lines RAW-BlueTM and THP1 BlueTM (Fig 2, *G*, and see Fig E9, *A–C* in this article's Online Repository at www.jacionline.org). These data suggest that, in accord with other reports,^{5, 10} enhanced targeting and presentation via AGE receptors rather than conventional DC maturation may be implicated in the increased immunogenicity of DR peanut antigens in vivo. The complex variety of AGE adducts and the diversity and redundancy of their receptors means that more study is required to elucidate the precise receptors and pathways implicated in DC uptake and presentation of peanut antigens to T cells.

This report provides the first in vivo support for the enhanced immunogenicity of DR peanuts, probably mediated by oxidation-driven generation of AGE-related adducts in peanut allergens. Two other major findings with clinical implications are: *i*) high reactivity of DR peanut-derived responses to raw peanut antigens suggesting that roasting may have a more important impact on the immunogenicity rather than antigenicity of peanuts at the priming step of sensitization, which could be further aggravated by subsequent exposure to raw antigens *ii*) cutaneous sensitization provoking further hypersensitivity in the gut upon oral antigen exposure. A better understanding of how high-temperature antigen modification, such as peanut dry roasting, leads to allergic sensitization should inform future preventive strategies including those concerning early age exposure, and therapeutic measures such as the choice and route of antigen delivery in desensitization strategies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

AGE	Advanced glycation end products
APC	Antigen presenting cell

BMDC	Bone marrow-derived dendritic cells
CPH	Crude peanut homogenate
FA	Food allergy
GI	Gastrointestinal
IC	Epicutaneous
LP	Lamina propria
LPS	Lipopolysaccharide
MLN	Mesenteric lymph node
PPE	Peanut protein extract
SC	Subcutaneous
Th2	T helper type 2

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FIG 1. Immunogenicity of raw and dry-roasted (DR) peanut extracts in BALB/c mice
A, SC-primed, post-IG gavage anti-raw PPE IgG and IgE, and **B**, IgE RBL degranulation.
C–D, ^3H thymidine incorporation and cytokines in raw PPE-pulsed MLN cell cultures from experiment in (**A**). **E**, Anti-raw PPE IgE responses to SC sensitization and raw peanut feeding. **F–H**, Anti-raw PPE antibody and RBL degranulation to direct GI sensitization. **I** and **J**, Anti-raw PPE antibody responses to EC sensitization. **K**, Eosinophils (arrow heads) in H&E staining of jejunum lamina propria. **L**, Flow cytometric analysis of disaggregated lamina propria (LP) and **M**, PPE-pulsed MLN cell cytokines.

FIG 2. AGE adducts in dry-roasted (DR) peanuts

A, Raw and DR Ara h1 recombinant receptor binding by ELISA. **B**, BMDC binding of fluoresceinated Ara h1 and its inhibition, by flow cytometry. **C**, DR or raw PPE competition for maleylated or AGE-HEL BMDC presentation to 3A9 hybridoma. **D** and **E**, PPE-pulsed BMDC surface marker and cytokine analysis by flow cytometry and Luminex®. **F**, PPE co-culture with HEL in BMDC presentation to 3A9 hybridoma. **H**, NFκB/AP1 activation in RAW-Blue™ cells pulsed with HEL, PPE or LPS ± polymixin B.