HHS Public Access

Author manuscript

J Allergy Clin Immunol. Author manuscript; available in PMC 2022 July 01.

Published in final edited form as:

J Allergy Clin Immunol. 2021 July ; 148(1): 182–194.e4. doi:10.1016/j.jaci.2020.11.047.

Peanut protein acts as a Th2 adjuvant by inducing RALDH2 in human antigen-presenting cells

Bert Ruiter, PhD[1,2,*], **Neal P. Smith, MS**[1], **Elizabeth Fleming, BS**[1], **Sarita U. Patil, MD**[1,2,3], **Barry K. Hurlburt, PhD**[4], **Soheila J. Maleki, PhD**[4], **Wayne G. Shreffler, MD, PhD**[1,2,3]

[1]Center for Immunology & Inflammatory Diseases, Massachusetts General Hospital, Boston, MA.

[2]Harvard Medical School, Boston, MA.

[3]Food Allergy Center, Massachusetts General Hospital, Boston, MA.

[4]US Department of Agriculture, Agricultural Research Service, New Orleans, LA.

Abstract

Background: Peanut is a potent inducer of pro-allergenic Th2 responses in susceptible individuals. Antigen-presenting cells (APC) including dendritic cells and monocytes instruct naïve T cells to differentiate into various effector cells, determining immune responses such as allergy and tolerance.

Objective: We sought to detect peanut protein (PN)-induced changes in gene expression in human myeloid dendritic cells (mDC) and monocytes, identify signaling receptors that mediate those changes, and assess how PN-induced genes in mDC impact their ability to promote T cell differentiation.

Methods: mDC, monocytes, and naïve $CD4^+$ T cells were isolated from blood bank donors and peanut-allergic patients. APC were incubated with PN and other stimulants, and gene expression was measured using microarray and RT-qPCR. To assess T cell differentiation, mDC were co-cultured with naïve Th cells.

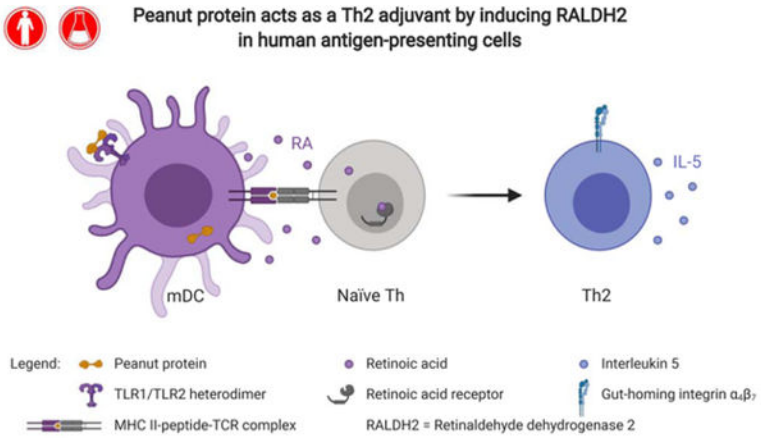
Results: PN induced a unique gene expression profile in mDC, including the gene that encodes retinaldehyde dehydrogenase 2 (RALDH2), a rate-limiting enzyme in the retinoic acid (RA)-producing pathway. Stimulation of mDC with PN also induced a 7-fold increase in enzymatic activity of RALDH2. Blocking antibodies against TLR1/TLR2, as well as siRNA targeting TLR1/TLR2, reduced expression of RALDH2 in PN-stimulated APC by 70%. Naïve Th cells co-cultured with PN-stimulated mDC showed an RA-dependent 4-fold increase in production of IL-5 and expression of integrin $\alpha_4\beta_7$.

*Corresponding author: Bert Ruiter, PhD, Massachusetts General Hospital, Center for Immunology & Inflammatory Diseases, Room 8.409, 149 13th Street, Boston, MA 02129, Phone: +1-617-726-6149, Fax: +1-617-726-5651, bruiter@mgh.harvard.edu.

Disclosure of potential conflict of interest: All authors declare that they have no competing interests.

Conclusions: PN induces RALDH2 in human APC by signaling through the TLR1/TLR2 heterodimer. This leads to production of RA, which acts on Th cells to induce IL-5 and gut-homing integrin. RALDH2 induction by PN in APC and RA-promoted Th2 differentiation could be an important factor determining allergic responses to peanut.

Graphical Abstract



Capsule summary:

Peanut protein induces expression and activity of RALDH2 in human antigen-presenting cells via TLR2, and retinoic acid produced by these cells promotes Th2 differentiation and gut-homing of naïve Th cells.

Keywords

Peanut allergy; peanut; dendritic cells; monocytes; RALDH2; TLR2; retinoic acid; Th2; IL-5; integrin $\alpha_4\beta_7$

Introduction

Peanut allergy currently affects approximately 1% of the US population, and its prevalence appears to be increasing[1]. Peanut is a potent inducer of pro-allergenic Th2 responses and peanut-specific IgE production in susceptible individuals[2–4]. Hence, the ratio of allergen-specific IgE to total IgE tends to be higher in peanut-allergic patients than in persons sensitized to other food or inhalant allergens[5]. Moreover, the symptoms of peanut allergy are relatively severe as compared to other food allergies, and peanut allergy is less frequently outgrown and more likely to occur at any age[6–8]. Dendritic cells (DC) are the key innate immune cells for antigen presentation and differentiation of naïve Th cells[9, 10]. Therefore, we hypothesize that direct DC activation by peanut protein may help explain the strongly polarized immune response to it.

Type 2 immunity and IgE are induced by extracellular pathogens such as parasitic helminths[11], as well as by noxious substances including bee venom[12, 13]. In addition, fungi such as *Alternaria* and *Aspergillus*, which can cause allergic disease and Th2-skewed inflammation, also contain molecules that activate DC to induce Th2 differentiation[14, 15].

These and other allergens and associated molecules have been observed to trigger Th2 immune responses by activating DC through protease-activated receptors, C-type lectin receptors (CLRs), and other mechanisms[16]. For example, glycans present in allergen extract from house dust mite activate DC by binding to the CLR Dectin-2, and induce the production of cysteinyl leukotrienes which promote Th2 responses[17]. Previous work from our group showed that the purified peanut glycoprotein allergen Ara h 1 binds to the CLR DC-SIGN, and activates human monocyte-derived DC (MoDC) to induce Th2 differentiation of naïve T cells[18].

Retinoic acid (RA) is a metabolite of vitamin A (retinol), and it plays various roles in immune responses[19]. Its production is regulated by an irreversible reaction catalyzed by retinal dehydrogenases (RALDHs), the expression of which is tightly regulated. Expression of the isoform RALDH2 occurs in leukocytes and can be induced in human basophils by IL-3[20]. In mice, the TLR2 agonist zymosan induces RALDH2 in splenic DC[21]. Furthermore, murine intestinal DC constitutively express RALDH2, which is induced by RA from gut epithelium and stromal cells[22–24]. Human gut-associated DC have also been shown to constitutively express RALDH2, and expression is increased in patients with Crohn's disease[25].

RA is a potent regulator of T cell differentiation[19]. In mice, RA produced by intestinal DC induces expression of the gut-homing receptors integrin $\alpha_4\beta_7$ and CCR9 on T cells[22]. Furthermore, RA promotes the TGF-β-mediated differentiation of peripheral $Foxp3^+$ regulatory T cells (Tregs) in murine models[26, 27]. Hence, RA may play an important role in the development and maintenance of oral tolerance. Induction of Tregs by RA in synergy with TGF-β has also been shown in human T cells in vitro[28]; however, RA without TGF-β induces differentiation of Th2 cells and enhances production of Th2 cytokines, in particular IL-5[29, 30]. A similar Th2-promoting and Th1-downregulating effect of RA was shown in murine T cells[31]. Adding to the complexity, other studies have shown that RA is essential for $CD4^+$ T cell immunity in general, including the maintenance of Th1 lineage stability[32, 33]. Therefore, regulation of T cell differentiation by RA appears to be dependent on accompanying signals, which decide whether a regulatory or effector response is promoted.

Our objective in the present study was to investigate the innate immunostimulatory properties of peanut protein extract (PN), and determine how these may contribute to peanut-induced Th2 immunity. We found that PN strongly induced expression of *ALDH1A2*, the gene encoding RALDH2, in human myeloid DC (mDC) and monocytes. In addition, we observed that PN signaled through the TLR1/TLR2 heterodimer to induce *ALDH1A2* expression, and this response was higher in monocytes from peanut-allergic patients than from tolerant subjects. Moreover, RA produced by PN-stimulated mDC acted in trans on co-cultured naïve $CD4^+$ T cells to induce expression of integrin $\alpha_4\beta_7$, reduce expression of the skin-homing receptor cutaneous lymphocyte-associated antigen (CLA), and enhance production of IL-5. These findings provide novel insight into the interplay between innate and adaptive immune responses to peanut protein, and elucidate a mechanism that may be relevant in allergy to peanut.

Methods

Cells

Human peripheral blood mononuclear cells (PBMC) were isolated by means of density gradient centrifugation (Ficoll-Paque Plus; GE Healthcare, Chicago, IL), from blood donor buffy coats (New York Blood Center, New York, NY, and Massachusetts General Hospital Blood Transfusion Service, Boston, MA), and from patient blood samples. The patients were recruited with informed consent, and the study was approved by the Institutional Review Board of Mass General Brigham (protocol no. 2010P002672). In these patients, peanut allergy was confirmed or ruled out by double-blind placebo-controlled food challenge at the Food Allergy Center at Massachusetts General Hospital. Myeloid DC and plasmacytoid DC were isolated from PBMC by using the CD1c (BDCA-1)$^+$ DC isolation kit and the Diamond (BDCA-4)$^+$ plasmacytoid DC isolation kit, respectively (Miltenyi Biotec, Bergisch Gladbach, Germany). Naïve CD4$^+$ T helper cells were isolated using the EasySep human naïve CD4$^+$ T cell isolation kit (Stemcell Technologies, Vancouver, British Columbia, Canada). Monocytes and basophils were isolated using the EasySep human CD14 positive selection kit, and EasySep human basophil isolation kit, respectively (Stemcell Technologies). All cells were cultured in StemSpan serum-free expansion medium (Stemcell Technologies) with penicillin (100 U/ml) and streptomycin (100 μg/ml) (Thermo Fisher, Waltham, MA).

Peanut protein extract and other allergens

Unsalted dry-roasted peanuts (Runner and Virginia cultivars) were shelled and ground to a smooth paste, which was defatted by washing with acetone. The acetone was drained using Whatman paper, and the defatted peanut paste was dried and pulverized into a powder. Protein was extracted by agitating the peanut powder with PBS containing protease inhibitor cocktail without EDTA (Roche, Basel, Switzerland). This suspension was cleared by centrifugation, and the supernatant (peanut protein extract, PN) was sterile-filtered before use in cell cultures. A low level of endotoxin (4 EU/mg, 0.4 ng/mg) was detected in the PN. However, in PN-coated culture plate wells, endotoxin contamination was below detection levels (<0.01 EU/ml, PyroGene assay; Lonza, Basel, Switzerland). A separate peanut extract made from raw peanuts (Florunner cultivar, obtained from Dr. Marshall Lamb, National Peanut Research Laboratory (NPRL), Dawson, GA) was used to isolate a peanut protein fraction (PN Fr.) with high *ALDH1A2*-inducing activity. This peanut extract was brought to 65% saturation with ammonium sulfate and cleared by centrifugation. The supernatant was brought to 100% saturation with ammonium sulfate and again cleared by centrifugation. The precipitate was dissolved in buffer A (65 mM Tris-Cl pH 7.0, 100 mM NaCl, 1 mM EDTA). Insoluble material was removed by filtration with Whatman paper and the solution was loaded onto a High S column (Bio-Rad, Hercules, CA). The column was washed with buffer A, and the PN Fr. was eluted with buffer B (65 mM Tris-Cl pH 7.0, 300 mM NaCl, 1 mM EDTA). Purified natural Ara h 1 and Ara h 2 were obtained from Indoor Biotechnologies (Charlottesville, VA). Soybean protein extract was from Dr. M. Masilamani, Icahn School of Medicine at Mount Sinai, New York, NY. Birch pollen, cockroach and cat dander allergen extracts were from Stallergenes Greer (Cambridge, MA). Cow's milk protein (skim milk powder) was from Millipore-Sigma (Burlington, MA).

Cell culture and gene expression analysis

Cell culture plates (48-well) were coated overnight with PN, PN Fr., or other indicated allergens at 50 μg/ml in carbonate-bicarbonate buffer (0.05M, pH 9.6, Millipore-Sigma) and washed once with PBS, before mDC or other cell types were added (2–5×10^5 cells/well) in StemSpan medium. The controls lipopolysaccharide (LPS, 100 ng/ml; Millipore-Sigma) and cholera toxin (CT, 1 μg/ml; List Biological Laboratories, Campbell, CA) were used in solution instead of coated. Cells were incubated for 8–24h at 37ºC, and total RNA was isolated using the RNeasy Plus Micro Kit (Qiagen, Hilden, Germany). For the gene array analysis, half of each RNA sample was used to make an RNA pool for each variable, including RNA from mDC of all blood donors in the experiment. This was the starting material for analysis with the GeneChip Human Genome U133 Plus 2.0 array (Affymetrix, Santa Clara, CA). GeneChip microarray assays were performed at the Microarray Shared Resource Facility at Icahn School of Medicine at Mount Sinai, New York, NY, according to the manufacturer's instructions. The other half of the RNA was used to synthesize cDNA (iScript cDNA synthesis kit; Bio-Rad). Quantitative PCR (qPCR) was performed by using gene-specific primers (PrimePCR SYBR Green Assay; Bio-Rad), SYBR green (iTaq Universal SYBR Green Supermix; Bio-Rad), and a StepOnePlus Real-Time PCR instrument (Applied Biosystems, Foster City, CA). RT-qPCR data were analyzed using the $2^{-(\Delta Ct)}$ method, which calculated expression of *ALDH1A2* or *IL1A* relative to the housekeeping gene *RPL41* or *B2M* for each variable, or using the $2^{-(\Delta\Delta Ct)}$ method, which calculated expression of *ALDH1A2* or *IL1A* in stimulated cells relative to expression of *ALDH1A2* or *IL1A* in unstimulated cells (fold change).

Analysis of gene array data

Gene microarray data analysis was performed using R software (version 3.6.1)[34]. Expression values were $\log_2$-normalized using the *RMA* function from the package *Affy* (version 1.62.0)[35]. All $\log_2$ fold change values were calculated by comparing the $\log_2$-normalized expression values in the stimulated samples to the $\log_2$-normalized expression values of the respective unstimulated samples. Heatmaps were rendered with the *gplots* package (version 3.0.3) using the function *heatmap.2*.

Assessment of RALDH2 enzymatic activity

Cell culture plates (48-well) were coated overnight with PN, before mDC and soluble stimuli (LPS, CT) were added as indicated. After 24h, cells were harvested using ice-cold staining buffer (PBS + 0.5% BSA + 2mM EDTA) to detach adherent mDC. Enzymatic activity of RALDH2 was assessed using the Aldefluor kit (Stemcell Technologies), according to the manufacturer's instructions. Cells were labeled with APC-conjugated anti-CD11c (clone B-ly6), PE-conjugated anti-HLA-DR (G46–6) (both from BD Biosciences, San Jose, CA), and Live/Dead Fixable Violet stain (L34955; Thermo Fisher), and analyzed with an LSRII flow cytometer (BD Biosciences).

Pattern recognition receptor ligand screen with PN Fr. and Ara h 1

HEK293-derived reporter cells expressing human TLRs or CLRs, or mouse TLR2, were incubated with PN Fr. or Ara h 1 (5 μg/ml each), or heat-killed *Listeria monocytogenes*

(HKLM, 1×10^6/ml), in HEK-Blue Detection medium for 16–24h in a 96-well plate, with 50,000 – 75,000 cells/well in 200 μl total volume, in triplicate (PRR ligand screening service, InvivoGen, San Diego, CA). Activation of TLRs and CLRs was determined by measuring expression and activity of secreted embryonic alkaline phosphatase (SEAP). The SEAP reporter is under the control of a promoter inducible by the transcription factor NF-κB. This reporter gene allows the monitoring of signaling through the TLR or CLR, based on the activation of NF-κB. SEAP was secreted by the cells grown in HEK-Blue Detection medium, and this medium changed to a purple/blue color in the presence of SEAP activity. The optical density was read at 650 nm on a SpectraMax 340PC absorbance detector (Molecular Devices, San Jose, CA).

TLR blocking experiments with mDC

Myeloid DC were pre-incubated with monoclonal neutralizing antibodies against human TLR1 (clone H2G2) and TLR2 (T2.5), or TLR2 and TLR6 (C5C8) (5 μg/ml each; InvivoGen) in StemSpan medium for 30 minutes at room temperature, before the cells were added to PN-coated culture plates and incubated for 16h. In separate experiments, mDC were added to PN Fr.- or PN-coated plates and the TLR2 inhibitor OxPAPC (30 μg/ml) or the TLR2/TLR6 agonist zymosan (25 μg/ml) (both from InvivoGen) were added as indicated, before the cells were incubated for 16h. RT-qPCR was performed and gene expression was analyzed as described above.

TLR knockdown experiments with monocytes

Monocytes were isolated as described above, and transfected with siRNA using the Human Monocyte Nucleofector kit (Lonza) and a Nucleofector II instrument (Amaxa/Lonza), according to the manufacturer's instructions. The TLR1, TLR2, MYD88, and non-targeting control (NT) siRNA pools (ON-TARGETplus SMARTpool; Horizon Discovery, Cambridge, UK) were used at 300 nM. Immediately following nucleofection, 0.5 ml of RPMI + 10% FBS was added to the cells, which were put into culture with an additional 1 ml of this medium and left to recover at 37°C for 72h. Cells were harvested and a subset was used to verify efficient knockdown via flow cytometry, by labeling with Biotin anti-TLR1 (TLR1.136), Streptavidin-PE (both from BioLegend, San Diego, CA), AF647-conjugated anti-TLR2 (11G7), FITC-conjugated CD14 (M5E2) (both from BD Biosciences), PE-Cy7-conjugated anti-TLR4 (HTA125), and Live/Dead Fixable Violet stain (both from Thermo Fisher). The remaining cells were added to PN Fr.-coated 48-well culture plates (5×10^5 cells/well), and incubated in StemSpan medium for 16h. The TLR1/TLR2 agonist Pam3CSK4 (1 μg/ml) and the TLR4 agonist LPS (Ultrapure; 100 ng/ml) (both from InvivoGen) were added as indicated. RT-qPCR was performed and gene expression was analyzed as described above.

Co-cultures of mDC and naïve T cells

Cell culture plates (48-well) were coated overnight with PN, before mDC were added (1×10^5/well). After 4h incubation, autologous naïve $CD4^+$ T cells were added (9×10^5/well), together with a low dose (1 pg/ml) of staphylococcal enterotoxin B (SEB; List Biological Laboratories) in all cultures, to provide TCR engagement and induce T cell activation. All-trans retinal (RAL; 25 nM), all-trans retinoic acid (RA; 2.5 nM), 4-

Diethylaminobenzaldehyde (DEAB; 10 μM) (all from Millipore-Sigma), and LE540 (1 μM; Wako Chemicals, Richmond, VA) were added in indicated cultures. Myeloid DC and T cells were co-cultured for 6 days, before supernatants were collected for cytokine measurement, and T cells were analyzed for surface marker expression. T cells were labeled with AF700-conjugated anti-CD3 (UCHT1), APC-Cy7-conjugated anti-CD4 (RPA-T4), BV605-conjugated anti-Cutaneous Lymphocyte Antigen (CLA; HECA-452) (all from BD Biosciences), FITC-conjugated anti-integrin β_7 (FIB504), PE-conjugated anti-integrin α_4 (CD49d; 9F10), and Live/Dead Fixable Violet stain (all from Thermo Fisher). Cytokine concentrations in supernatants were measured using Cytometric Bead Array (IL-4, IL-5, IL-9, IL-10, IL-13, IFN-γ; BD Biosciences), according to the manufacturer's instructions. Flow cytometry was performed with an LSRII instrument and data were analyzed using FlowJo software (BD Biosciences).

Statistical analysis

Prism 7 (GraphPad Software, San Diego, CA) and R (version 3.6.1) were used for statistical analysis. We used the D'Agostino-Pearson omnibus normality test or the Shapiro-Wilk normality test to assess for normal distribution. The specific parametric and nonparametric statistical tests are indicated in the figure legends.

Data availability

The GeneChip microarray datasets generated in the course of this project have been deposited at the National Center for Biotechnology Information Gene Expression Omnibus (GEO) database, under accession number GSE159706.

Results

PN induces a unique gene expression profile in human mDC, including the upregulation of *ALDH1A2*

To evaluate the innate immunostimulatory potential of PN, mDC were cultured with PN, as well as various other allergens and known mDC stimuli. In a pilot experiment, mDC from 4 non-selected blood bank donors were cultured for 8h and 24h with and without PN (Fig. E1A, Data file E1). The 24h time point proved to be most informative for monitoring changes in expression of immune-related genes. Moreover, a previous study suggested that the 24h time point is optimal for analysis of early sustained, middle sustained, and late gene expression in human DC[36]. Therefore, in the main experiment, mDC from 7 non-atopic blood bank donors were incubated for 24h with PN, the purified peanut allergens Ara h 1 and Ara h 2, and extracts from other food and inhalant allergens (Fig. 1A, Data file E2). Lipopolysaccharide (LPS) and cholera toxin (CT) were included as known adjuvants, respectively inducing Th1- and Th2-skewing properties in human DC[37, 38]. The included 7 subjects had no detectable serum IgE specific for PN or any of the other allergens, to exclude the possibility of allergen binding and signaling through the high affinity IgE receptor, FcεRI.

PN induced a unique gene expression profile, with 185 genes upregulated and 195 genes downregulated 2-fold or more (Fig. 1A, Data file E2). PN was the most potent of all tested

allergens in inducing changes in mDC gene expression. The expression profile induced by PN showed little similarity with the other allergens, but there was substantial overlap with LPS-induced gene expression, consistent with overlap in signaling pathway activation. This was not due to endotoxin contamination of PN, since endotoxin levels in PN-coated wells were below the detection limit. Furthermore, several canonical genes that are strongly induced by LPS, such as *IL12B*, *IL6* and *IL1A*, were not enhanced by PN (Fig. 1B, Data file E1 and E2).

The most prominent gene uniquely upregulated by PN in mDC was *ALDH1A2*, which encodes the rate-limiting RA-producing enzyme retinaldehyde dehydrogenase 2 (RALDH2) (Fig. 1A and E1A). This gene was induced by PN after 8h (18-fold) and 24h (23-fold) in the pilot, and after 24h (9-fold) in the main experiment (Data file E1 and E2). We observed that in addition to *ALDH1A2*, the induction of other highly differentially expressed genes by PN was consistent between the pilot and the main experiment (Fig. E1B). For the latter experiment, the upregulation of *ALDH1A2* was confirmed by RT-qPCR in the 7 individual donors (median 35-fold increase, $P < 0.05$) (Fig. 1C). No other stimulus induced more than 3-fold up- or downregulation of *ALDH1A2* expression.

To examine whether PN induced *ALDH1A2* in cell types other than mDC, the response to PN was also measured in plasmacytoid DC (pDC), monocytes, naïve $CD4^+$ T cells, and basophils from 4 additional nonselected blood donors. PN was observed to upregulate *ALDH1A2* expression only in mDC and monocytes (Fig. 1D).

PN increases enzymatic activity of RALDH2 in mDC

To investigate whether the robust upregulation of *ALDH1A2* gene expression resulted in increased activity of RALDH2 in mDC, the activity of this enzyme was assessed using the Aldefluor assay (see Methods) (Fig. 2). This assay makes use of a fluorescent substrate that freely diffuses into cells, but becomes negatively charged upon conversion by RALDH. This prevents the substrate from leaving the cells, and allows for analyzing RALDH activity by flow cytometry. Treatment of mDC with PN resulted in a profound increase in $Aldefluor^+$ mDC frequencies as compared with unstimulated mDC (median 15% vs 2%, $P < 0.05$), indicating an increase in RALDH2 enzymatic activity (Fig. 2B and C). This effect was completely blocked by the RALDH-inhibitor diethylaminobenzaldehyde (DEAB). RALDH2 activity was not induced by LPS or CT, which confirmed the results obtained with gene array and RT-qPCR.

PN signals through the TLR1/TLR2 heterodimer to induce expression of *ALDH1A2* in human mDC and monocytes

After observing the strong induction of *ALDH1A2* in mDC and monocytes by PN, we hypothesized that one or multiple innate immune receptors were responsible for this effect. A previous report had shown that the yeast cell wall derivative zymosan strongly induced expression of *ALDH1A2* in murine splenic DC via activation of TLR2 and Dectin-1[21]. We performed a pattern recognition receptor (PRR) ligand screen with a fraction of PN (PN Fr., see Methods), which was enriched for *ALDH1A2*-inducing activity. PN Fr. was observed to signal through TLR2, but not through Dectin-1 or any other tested extracellular human PRR

(Fig. 3A). Furthermore, PN Fr. activated human TLR2 more strongly than murine TLR2. In contrast, the peanut allergen Ara h 1, which was shown previously to activate multiple PRRs[18, 39, 40], but failed to induce *ALDH1A2*, did not signal through TLR2 (Fig. 3B and C).

TLR1 and TLR6, the two receptors that form a heterodimer with TLR2, were not included in the screen. To assess whether PN signals through the TLR1/TLR2 or TLR2/TLR6 heterodimer, mDC were pre-incubated with blocking antibodies against these TLRs before stimulating them with PN (Fig. 3D-F and Fig. E2A-C). Induction of *ALDH1A2* by PN was lower in mDC treated with blocking antibodies against TLR1 and TLR2 than in untreated mDC (Fig. 3E; median 12-fold vs 29-fold induction, $P < 0.01$). Treatment with blocking antibodies against TLR2 and TLR6 also inhibited the induction of *ALDH1A2* by PN, but the blocking was not as effective as with anti-TLR1 and -TLR2 antibodies (Fig. E2B and C).

To confirm that PN induces *ALDH1A2* via TLR1/TLR2, we inhibited expression of these receptors, as well as the universal TLR adapter protein MyD88, by using specific siRNA in monocytes (Fig. 3G-I and Fig. E2D-J). Knockdown of TLR1 and TLR2 resulted in approximately 50% reduction of TLR1 and TLR2 on the cell surface, whereas TLR4 was unaffected (Fig. E2J). Treatment with TLR1- and TLR2-specific siRNA, as well as with MyD88-specific siRNA, inhibited the induction of *ALDH1A2* by PN Fr. as compared to monocytes treated with non-targeting siRNA (Fig. 3H; median 3.6-fold and 4.2-fold vs 11.2-fold induction, $P < 0.05$). The specificity of the knockdown of TLR1 and TLR2 was confirmed by stimulating the monocytes with the TLR1/TLR2 agonist Pam3CSK4 and the TLR4 agonist LPS. Whereas the induction of the proinflammatory gene *IL1A* by Pam3CSK4 was inhibited in monocytes treated with TLR1- and TLR2-specific siRNA, the *IL1A* response to LPS remained unaffected. In contrast, treatment of monocytes with MyD88-specific siRNA inhibited the induction of *IL1A* by both Pam3CSK4 and LPS (Fig. E2D-I).

Lastly, we tested whether the PN-induced *ALDH1A2* response in mDC was decreased by the TLR2 inhibitor OxPAPC (Fig. 3J-L). In the presence of OxPAPC, the induction of *ALDH1A2* by PN Fr. was inhibited as compared to mDC without OxPAPC (Fig. 3K; median 66-fold vs 1288-fold induction, $P < 0.05$).

In human mDC and monocytes, PN appears to be a remarkably potent inducer of *ALDH1A2*. We observed that the TLR2 agonists zymosan and Pam3CSK4 also induced expression of *ALDH1A2*, but to a lower extent than PN (Fig. 4A and B). In contrast, PN is a poor inducer of *IL1A*, whereas Pam3CSK4 triggers a strong response (Fig. 4C). Based on these data, we suspect that in addition to TLR1/TLR2 heterodimer, another receptor or signaling pathway may be involved in the innate immune sensing of PN and the induction of *ALDH1A2*.

In sum, these results imply that PN induces expression of *ALDH1A2* at least in part by signaling through the TLR1/TLR2 heterodimer.

PN-induced *ALDH1A2* expression is increased in monocytes from peanut-allergic patients

Innate immune sensing of PN may be an important factor in driving the type 2 immune responses that result in sensitization and allergic reactions to peanut in susceptible individuals. To study whether the *ALDH1A2* response to PN differed between peanut-allergic and tolerant subjects, we compared the PN-induced expression of *ALDH1A2* in monocytes from peanut-allergic patients and individuals who were atopic, but clinically tolerant to peanut (Fig. E3). We found that induction of *ALDH1A2* by PN Fr. was higher in peanut-allergic than in tolerant subjects (Fig. E3B; median 11.1-fold vs 4.7-fold induction, $P < 0.05$). In contrast, Pam3CSK4-induced expression of *IL1A* was not different between the groups, indicating that there was no fundamental difference in signaling through the TLR1/TLR2 heterodimer (Fig. E3D).

RA produced by PN-stimulated mDC induces integrin $\alpha_4\beta_7$ and IL-5 in naïve $CD4^+$ T cells

Increased RALDH2 activity in mDC could lead to higher production of RA, which has been implicated in various aspects of T cell differentiation[19]. To investigate the effect of PN-induced RA production on differentiation of T cells, mDC treated with or without PN were co-cultured with autologous naïve $CD4^+$ T cells. Cells were cultured with medium alone or in the presence of the RA precursor and RALDH2 substrate retinal (RAL), whereas RA was used as a positive control. Expression of the gut-homing integrin $\alpha_4\beta_7$ on the T cells, which had been shown previously to be RA-sensitive[20, 22], was measured to study the effect of newly produced RA by the mDC. As is shown in Fig. 5A and B, baseline expression of integrin $\alpha_4\beta_7$ in co-cultured T cells was low, whereas it was strongly induced by exogenous RA. Addition of RAL enhanced expression of integrin $\alpha_4\beta_7$ more on T cells co-cultured with PN-treated mDC than with unstimulated mDC (median 27% vs 10% integrin $\alpha_4\beta_7^+$ T cells, $P < 0.001$), which indicated an increased activity of RALDH2 (Fig. 5B). In contrast, we observed that expression of the skin-homing receptor cutaneous lymphocyte-associated antigen (CLA) was decreased on T cells in co-cultures with RAL and RA (Fig. E4A and B). Inhibition of CLA expression on T cells by RA has been previously reported as well[41]. Addition of RAL to the co-cultures decreased expression of CLA more on T cells cultured with PN-treated mDC than with unstimulated mDC (median 8% vs 38% CLA^+ T cells, $P < 0.01$), which again indicated an increased activity of RALDH2 and enhanced RA production in PN-stimulated mDC (Fig. E4B).

In addition to surface markers, T cell cytokines were measured, in supernatants of the co-cultures. We observed that addition of RA increased the production of IL-5, but tended to suppress the release of IL-13 and IFN-γ (Fig. 5D, F, G). Production of IL-4 was below the detection limit, whereas IL-9 was slightly decreased by RA, and IL-10 was not affected by RA (data not shown). Addition of RAL resulted in higher production of IL-5 by T cells co-cultured with PN-treated mDC than with unstimulated mDC (Fig. 5D; median 53 vs 12 pg/ml, $P < 0.01$). Interestingly, PN-treated mDC stimulated production of IL-5 as well as IL-13 in co-cultured T cells even in the absence of RAL or RA, which confirmed that PN has additional Th2-skewing adjuvant properties unrelated to the induction of RALDH2[18] (Fig. 5D and F).

To validate that the increase in expression of integrin $\alpha_4\beta_7$ and production of IL-5 in T cells co-cultured with PN-stimulated mDC and RAL was dependent on RALDH2 activity and RA, we added either the RALDH inhibitor DEAB or the retinoic acid receptor (RAR) antagonist LE540 to the cultures (Fig. 5C and E). Addition of DEAB as well as LE540 resulted in significantly lower expression of integrin $\alpha_4\beta_7$ and production of IL-5, indicating that these responses were dependent on RALDH2 activity in PN-stimulated mDC and RA signaling through RAR in the co-cultured T cells.

Together, these results indicate that PN-stimulated mDC have increased capacity to metabolize RAL into RA, which can act directly on naïve Th cells to induce expression of the gut-homing integrin $\alpha_4\beta_7$, reduce expression of the skin-homing receptor CLA, and enhance the production of IL-5.

Discussion

The various roles of RA in the immune system have been intensively studied in the past two decades. RA is now known to be highly important in T cell and B cell differentiation, as well as antigen-presenting cell function[19]. Here, we demonstrate that peanut protein induces expression and activity of RALDH2 in human monocytes and mDC. These mDC produce RA, which increases expression of the gut-homing integrin $\alpha_4\beta_7$ on $CD4^+$ T cells, decreases the skin-homing receptor CLA, and enhances production of the Th2 cytokine IL-5. All these features are relevant in allergy to peanut, as similarly activated T cells *in vivo* will migrate to the gastrointestinal tract and display Th2 effector functions upon encounter of antigen. Numerous studies have shown a Th2-promoting effect of RA in mice, both *in vivo*[42] and *in vitro*[31], as well as in humans[20, 29, 30, 43], with increased production of IL-4, IL-5, and in some cases IL-13. We observed enhanced RA-dependent production of IL-5 by T cells co-cultured with PN-stimulated mDC, but no additional RA-induced production of IL-13. Levels of IL-4 were below the detection limit of our assay, although this does not rule out the possibility that low quantities of cytokine were produced locally and consumed by T cells in an autocrine manner[44]. Our data indicate that RA, in the absence of co-factors such as TGF-β, preferentially induces differentiation of Th2 cells. We previously reported that RA in synergy with IL-2 triggers production of IL-5 and IL-13 and expression of integrin $\alpha_4\beta_7$ in human ILC2 cells[45]. Hence, mDC and monocytes producing RA upon activation by peanut protein may promote type 2 immune responses by acting on both T cells and ILC2 cells.

Baseline expression of RALDH2 in human peripheral blood mDC is very low, which supports the notion that production of RA by RALDH2 is under tight control. Various pathogen-derived and other components have been identified that induce RALDH2 expression in human DC. The PPARγ agonist rosiglitazone triggers a ~4-fold increase in *ALDH1A2* expression in MoDC[46], and RA itself induces a ~4-fold increase in RALDH2 activity in this cell type[47]. Furthermore, *Aspergillus*-derived proteases as well as *S. mansoni*-derived soluble egg antigen (SEA) generate a ~10-fold increase in expression of *ALDH1A2* in MoDC[48, 49]. In addition, *Bifidobacterium infantis* was shown to induce a ~6-fold increase in *ALDH1A2* expression in mDC[50], and similar results were observed when mDC were stimulated with vitamin D_3 in the presence of GM-CSF[51]. In light of these findings, it is

remarkable that peanut protein, a ubiquitous source of dietary protein around the world, induces a >10-fold increase in *ALDH1A2* in human mDC. We found that PN induces expression of *ALDH1A2* at least in part by signaling through TLR2, which is the receptor that also mediates *ALDH1A2* induction by the microbial antigens zymosan[21], *S. mansoni* SEA[49], and *B. infantis*[50]. This raised the concern that a contaminant such as aflatoxin[52] or endotoxin, rather than peanut protein itself, was responsible for its *ALDH1A2*-inducing effect. To answer this, we stimulated mDC with aflatoxin B1 (data not shown) or LPS, but we did not detect induction of *ALDH1A2* by these components.

Whole peanut protein is a complex mixture consisting of the known allergens Ara h 1–17, as well as numerous other proteins[53]. It is possible that the innate immunostimulatory activity of PN originates from known allergens as well as non-allergenic proteins, which may act as a local Th2-skewing adjuvant and promote IgE responses to the allergens in the same food matrix. We have attempted to identify the *ALDH1A2*-inducing component in PN, and found that neither Ara h 1, Ara h 2, Ara h 3, Ara h 6, or Ara h 8, nor a combination of these proteins, was responsible for this activity. Enzymatic digestion of PN with pepsin or trypsin abrogated its *ALDH1A2*-inducing activity, but deglycosylation did not (data not shown). By fractionation of PN, we were able to isolate fractions with enhanced activity such as PN Fr., which was used for several experiments in this study. We have, however, not yet been able to identify the individual peanut protein(s) responsible for the induction of *ALDH1A2*. Multiple fractionation and detection techniques will need to be applied to obtain the resolution necessary to isolate this protein or these proteins, and this will be the goal of further studies.

An important question is whether induction of RALDH2 activity in human APC by peanut protein may occur *in vivo*, and have immunological consequences. The gastrointestinal tract is specialized in inducing and maintaining tolerance to dietary antigens[54]. In mice, intestinal epithelial cells and gut-associated DC constitutively express RALDH[22, 26], and RALDH expression has also been shown in the human intestine[55]. Hence, the environment is rich in RA, which acts synergistically with TGF-β to induce Tregs[26, 27]. Upon ingestion, peanut protein may induce RALDH2 in gut-associated DC similar to our findings in mDC. In the immunological context of the gut, an increase in RA production by gut-resident DC may stimulate differentiation of Tregs rather than Th2 cells. As the gastrointestinal tract promotes tolerance to food antigens by default, alternate routes of exposure, such as the skin, have been hypothesized to play a role in sensitization to peanut. This hypothesis was first based on the observation that peanut allergy in children correlated with the use of skin preparations containing peanut oil[56]. Furthermore, infants are frequently observed to be sensitized to peanut without apparent previous ingestion, and studies have shown that whereas early oral exposure to peanut is protective[57, 58], high-dose environmental (non-oral) exposure is a risk factor for developing peanut allergy, especially in children with an impaired skin barrier[59–61]. Elegant studies in mice have provided evidence for an important role of the skin as well as the respiratory tract in sensitization to peanut[62–64]. It is tempting to speculate that exposure to peanut protein via the skin or lungs induces RALDH2 in tissue-resident DC. After migrating to draining lymph nodes, these DC may produce RA in the absence of TGF-β, thereby promoting Th2 differentiation and gut homing of naïve peanut-specific Th cells.

It would be of interest to study the RALDH2-inducing effect of peanut protein in APC and its role in sensitization and allergy to peanut in animal models. To this end, we have stimulated various types of murine APC with PN *in vitro*. We did, however, not detect an induction of *ALDH1A2* expression in bone marrow-derived DC, skin DC, skin-draining lymph node DC, mesenteric lymph node DC, splenic DC, or peritoneal macrophages, from C57BL/6 as well as BALB/c mice (data not shown). This lack of response may be explained by the observation that PN Fr. did not activate mouse TLR2 as strongly as human TLR2, as is shown in Fig. 3B and C. Therefore, investigating the induction of RALDH2 in APC by peanut protein, and its immunological consequences, may be more fruitful in transgenic mice or other model systems.

ALDH1A2 is part of a specifically and reproducibly induced gene set by PN in mDC, which also includes *CCL22* (see Fig. 1A and Fig. E1). We confirmed this observation by measuring levels of CCL22 protein in supernatants from similar cultures, and found that production of this chemokine was significantly upregulated in mDC stimulated with PN Fr. (data not shown). CCL22 is known to play an important role in recruitment of Th2 cells, which express its receptor CCR4, and it has been implicated in Th2-driven disorders such as atopic dermatitis, asthma, and allergic rhinitis[65]. Moreover, a recent study suggested that CCL22 also promotes the differentiation of human Th2 cells[66]. As we observed that PN has additional Th2-skewing adjuvant properties besides the induction of RALDH2 in APC and their production of RA (see Fig. 5D and F), it is possible that the upregulation of CCL22 is one of those qualities. It would be interesting to explore the possibility that increased expression of RALDH2 and CCL22 by peanut protein-stimulated APC act in synergy to stimulate the differentiation as well as the recruitment of Th2 cells. While we considered this to be beyond the scope of this paper, it is an attractive subject for future studies.

In sum, we have discovered a novel mechanism by which peanut protein stimulates human APC and acts as a Th2-promoting adjuvant. These findings provide additional insight into the interplay between innate and adaptive immune responses to peanut protein, and may help explain the potent allergenicity of peanut in susceptible children and adults.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We would like to thank Stans den Hartog Jager, Els van Hoffen, Edward Knol, Terry Means, Ana Belén Blázquez, Cecilia Berin, Laverne Brown, Michiko Oyoshi, Hasmik Keshishian, Peggy Ozias-Akins, Brinda Monian, Daniel O'Connell, Mukund Varma, Simone Nish, Patrick Brennan, David Millar, and Sandra Denery for their valuable contributions to this project. In addition, we would like to acknowledge our colleagues at the MGH Department of Pathology Flow and Image Cytometry Research Core for their help with flow cytometry. The Flow Core obtained funding from the NIH Shared Instrumentation program with grants 1S10OD012027-01A1, 1S10OD016372-01, 1S10RR020936-01, and 1S10RR023440-01A1.

Funding:

This work was supported by the NIH/NIAID (grant K08 AI067722 and K08 AI067722-03S1 to W.G.S., and U19 AI095261 to W.G.S. and Andrew D. Luster), the US Department of Agriculture, Agricultural Research Service (USDA-ARS-6054-43440-046-00D to B.K.H and S.J.M), the Food Allergy Science Initiative (FASI), and institutional funds from Massachusetts General Hospital.

Abbreviations:

ALDH1A2	Aldehyde dehydrogenase 1 family, member A2
APC	Antigen-presenting cells
CCL22	C-C motif chemokine ligand 22
CLA	Cutaneous lymphocyte-associated antigen
CLR	C-type lectin receptor
CT	Cholera toxin
DEAB	Diethylaminobenzaldehyde
IFN-γ	Interferon-γ
LPS	Lipopolysaccharide
mDC	Myeloid dendritic cells
MoDC	Monocyte-derived dendritic cells
PBMC	Peripheral blood mononuclear cells
pDC	Plasmacytoid dendritic cells
PN	Peanut protein extract
PRR	Pattern recognition receptor
RA	Retinoic acid
RAL	Retinal
RALDH2	Retinaldehyde dehydrogenase 2
RAR	Retinoic acid receptor
RT-qPCR	Reverse transcription quantitative polymerase chain reaction
siRNA	Small interfering RNA
TGF-β	Transforming growth factor-β
TLR	Toll-like receptor

References

1. Sicherer SH, Sampson HA. Food allergy: A review and update on epidemiology, pathogenesis, diagnosis, prevention, and management. J Allergy Clin Immunol 2018; 141:41–58. [PubMed: 29157945]
2. Turcanu V, Maleki SJ, Lack G. Characterization of lymphocyte responses to peanuts in normal children, peanut-allergic children, and allergic children who acquired tolerance to peanuts. J Clin Invest 2003; 111:1065–72. [PubMed: 12671056]

3. Chiang D, Chen X, Jones SM, Wood RA, Sicherer SH, Burks AW, et al. Single-cell profiling of peanut-responsive T cells in patients with peanut allergy reveals heterogeneous effector TH2 subsets. J Allergy Clin Immunol 2018; 141:2107–20. [PubMed: 29408715]

4. Ruiter B, Smith NP, Monian B, Tu AA, Fleming E, Virkud YV, et al. Expansion of the CD4(+) effector T-cell repertoire characterizes peanut-allergic patients with heightened clinical sensitivity. J Allergy Clin Immunol 2020; 145:270–82. [PubMed: 31654649]

5. Hamilton RG, MacGlashan DW Jr., Saini SS. IgE antibody-specific activity in human allergic disease. Immunol Res 2010; 47:273–84. [PubMed: 20066506]

6. Bock SA, Munoz-Furlong A, Sampson HA. Further fatalities caused by anaphylactic reactions to food, 2001–2006. J Allergy Clin Immunol 2007; 119:1016–8. [PubMed: 17306354]

7. Gupta RS, Warren CM, Smith BM, Jiang J, Blumenstock JA, Davis MM, et al. Prevalence and Severity of Food Allergies Among US Adults. JAMA Netw Open 2019; 2:e185630. [PubMed: 30646188]

8. Sicherer SH, Sampson HA. Food allergy. J Allergy Clin Immunol 2010; 125:S116–25. [PubMed: 20042231]

9. Eisenbarth SC. Dendritic cell subsets in T cell programming: location dictates function. Nat Rev Immunol 2019; 19:89–103. [PubMed: 30464294]

10. Schuijs MJ, Hammad H, Lambrecht BN. Professional and 'Amateur' Antigen-Presenting Cells In Type 2 Immunity. Trends Immunol 2019; 40:22–34. [PubMed: 30502024]

11. Harris NL, Loke P. Recent Advances in Type-2-Cell-Mediated Immunity: Insights from Helminth Infection. Immunity 2017; 47:1024–36. [PubMed: 29262347]

12. Palm NW, Rosenstein RK, Yu S, Schenten DD, Florsheim E, Medzhitov R. Bee venom phospholipase A2 induces a primary type 2 response that is dependent on the receptor ST2 and confers protective immunity. Immunity 2013; 39:976–85. [PubMed: 24210353]

13. Palm NW, Rosenstein RK, Medzhitov R. Allergic host defences. Nature 2012; 484:465–72. [PubMed: 22538607]

14. Kobayashi T, Iijima K, Radhakrishnan S, Mehta V, Vassallo R, Lawrence CB, et al. Asthma-related environmental fungus, Alternaria, activates dendritic cells and produces potent Th2 adjuvant activity. J Immunol 2009; 182:2502–10. [PubMed: 19201906]

15. Lamhamedi-Cherradi SE, Martin RE, Ito T, Kheradmand F, Corry DB, Liu YJ, et al. Fungal proteases induce Th2 polarization through limited dendritic cell maturation and reduced production of IL-12. J Immunol 2008; 180:6000–9. [PubMed: 18424720]

16. Ruiter B, Shreffler WG. Innate immunostimulatory properties of allergens and their relevance to food allergy. Semin Immunopathol 2012; 34:617–32. [PubMed: 22886110]

17. Barrett NA, Rahman OM, Fernandez JM, Parsons MW, Xing W, Austen KF, et al. Dectin-2 mediates Th2 immunity through the generation of cysteinyl leukotrienes. J Exp Med 2011; 208:593–604. [PubMed: 21357742]

18. Shreffler WG, Castro RR, Kucuk ZY, Charlop-Powers Z, Grishina G, Yoo S, et al. The major glycoprotein allergen from Arachis hypogaea, Ara h 1, is a ligand of dendritic cell-specific ICAM-grabbing nonintegrin and acts as a Th2 adjuvant in vitro. J Immunol 2006; 177:3677–85. [PubMed: 16951327]

19. Larange A, Cheroutre H. Retinoic Acid and Retinoic Acid Receptors as Pleiotropic Modulators of the Immune System. Annu Rev Immunol 2016; 34:369–94. [PubMed: 27168242]

20. Spiegl N, Didichenko S, McCaffery P, Langen H, Dahinden CA. Human basophils activated by mast cell-derived IL-3 express retinaldehyde dehydrogenase-II and produce the immunoregulatory mediator retinoic acid. Blood 2008; 112:3762–71. [PubMed: 18495959]

21. Manicassamy S, Ravindran R, Deng J, Oluoch H, Denning TL, Kasturi SP, et al. Toll-like receptor 2-dependent induction of vitamin A-metabolizing enzymes in dendritic cells promotes T regulatory responses and inhibits autoimmunity. Nat Med 2009; 15:401–9. [PubMed: 19252500]

22. Iwata M, Hirakiyama A, Eshima Y, Kagechika H, Kato C, Song SY. Retinoic acid imprints gut-homing specificity on T cells. Immunity 2004; 21:527–38. [PubMed: 15485630]

23. Molenaar R, Knippenberg M, Goverse G, Olivier BJ, de Vos AF, O'Toole T, et al. Expression of retinaldehyde dehydrogenase enzymes in mucosal dendritic cells and gut-draining lymph node

stromal cells is controlled by dietary vitamin A. J Immunol 2011; 186:1934–42. [PubMed: 21220692]

24. Vicente-Suarez I, Larange A, Reardon C, Matho M, Feau S, Chodaczek G, et al. Unique lamina propria stromal cells imprint the functional phenotype of mucosal dendritic cells. Mucosal Immunol 2015; 8:141–51. [PubMed: 24938743]
25. Sanders TJ, McCarthy NE, Giles EM, Davidson KL, Haltalli ML, Hazell S, et al. Increased production of retinoic acid by intestinal macrophages contributes to their inflammatory phenotype in patients with Crohn's disease. Gastroenterology 2014; 146:1278–88 e1–2. [PubMed: 24503130]
26. Coombes JL, Siddiqui KR, Arancibia-Carcamo CV, Hall J, Sun CM, Belkaid Y, et al. A functionally specialized population of mucosal CD103+ DCs induces Foxp3+ regulatory T cells via a TGF-beta and retinoic acid-dependent mechanism. J Exp Med 2007; 204:1757–64. [PubMed: 17620361]
27. Mucida D, Park Y, Kim G, Turovskaya O, Scott I, Kronenberg M, et al. Reciprocal TH17 and regulatory T cell differentiation mediated by retinoic acid. Science 2007; 317:256–60. [PubMed: 17569825]
28. Wang J, Huizinga TW, Toes RE. De novo generation and enhanced suppression of human CD4+CD25+ regulatory T cells by retinoic acid. J Immunol 2009; 183:4119–26. [PubMed: 19717521]
29. Dawson HD, Collins G, Pyle R, Key M, Weeraratna A, Deep-Dixit V, et al. Direct and indirect effects of retinoic acid on human Th2 cytokine and chemokine expression by human T lymphocytes. BMC Immunol 2006; 7:27. [PubMed: 17118196]
30. Wansley DL, Yin Y, Prussin C. The retinoic acid receptor-alpha modulators ATRA and Ro415253 reciprocally regulate human IL-5+ Th2 cell proliferation and cytokine expression. Clin Mol Allergy 2013; 11:4. [PubMed: 24314292]
31. Iwata M, Eshima Y, Kagechika H. Retinoic acids exert direct effects on T cells to suppress Th1 development and enhance Th2 development via retinoic acid receptors. Int Immunol 2003; 15:1017–25. [PubMed: 12882839]
32. Brown CC, Esterhazy D, Sarde A, London M, Pullabhatla V, Osma-Garcia I, et al. Retinoic acid is essential for Th1 cell lineage stability and prevents transition to a Th17 cell program. Immunity 2015; 42:499–511. [PubMed: 25769610]
33. Hall JA, Cannons JL, Grainger JR, Dos Santos LM, Hand TW, Naik S, et al. Essential role for retinoic acid in the promotion of CD4(+) T cell effector responses via retinoic acid receptor alpha. Immunity 2011; 34:435–47. [PubMed: 21419664]
34. R Core Team (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/..
35. Gautier L, Cope L, Bolstad BM, Irizarry RA. affy--analysis of Affymetrix GeneChip data at the probe level. Bioinformatics 2004; 20:307–15. [PubMed: 14960456]
36. Huang Q, Liu D, Majewski P, Schulte LC, Korn JM, Young RA, et al. The plasticity of dendritic cell responses to pathogens and their components. Science 2001; 294:870–5. [PubMed: 11679675]
37. Hilkens CM, Kalinski P, de Boer M, Kapsenberg ML. Human dendritic cells require exogenous interleukin-12-inducing factors to direct the development of naive T-helper cells toward the Th1 phenotype. Blood 1997; 90:1920–6. [PubMed: 9292525]
38. Gagliardi MC, Sallusto F, Marinaro M, Langenkamp A, Lanzavecchia A, De Magistris MT. Cholera toxin induces maturation of human dendritic cells and licences them for Th2 priming. Eur J Immunol 2000; 30:2394–403. [PubMed: 10940931]
39. Royer PJ, Emara M, Yang C, Al-Ghouleh A, Tighe P, Jones N, et al. The mannose receptor mediates the uptake of diverse native allergens by dendritic cells and determines allergen-induced T cell polarization through modulation of IDO activity. J Immunol 2010; 185:1522–31. [PubMed: 20610655]
40. Mueller GA, Maleki SJ, Johnson K, Hurlburt BK, Cheng H, Ruan S, et al. Identification of Maillard reaction products on peanut allergens that influence binding to the receptor for advanced glycation end products. Allergy 2013; 68:1546–54. [PubMed: 24266677]

41. Yamanaka K, Dimitroff CJ, Fuhlbrigge RC, Kakeda M, Kurokawa I, Mizutani H, et al. Vitamins A and D are potent inhibitors of cutaneous lymphocyte-associated antigen expression. J Allergy Clin Immunol 2008; 121:148–57 e3. [PubMed: 17910894]
42. Broadhurst MJ, Leung JM, Lim KC, Girgis NM, Gundra UM, Fallon PG, et al. Upregulation of retinal dehydrogenase 2 in alternatively activated macrophages during retinoid-dependent type-2 immunity to helminth infection in mice. PLoS Pathog 2012; 8:e1002883. [PubMed: 22927819]
43. Lovett-Racke AE, Racke MK. Retinoic acid promotes the development of Th2-like human myelin basic protein-reactive T cells. Cell Immunol 2002; 215:54–60. [PubMed: 12142036]
44. Demeure CE, Yang LP, Byun DG, Ishihara H, Vezzio N, Delespesse G. Human naive CD4 T cells produce interleukin-4 at priming and acquire a Th2 phenotype upon repetitive stimulations in neutral conditions. Eur J Immunol 1995; 25:2722–5. [PubMed: 7589153]
45. Ruiter B, Patil SU, Shreffler WG. Vitamins A and D have antagonistic effects on expression of effector cytokines and gut-homing integrin in human innate lymphoid cells. Clin Exp Allergy 2015; 45:1214–25. [PubMed: 25959810]
46. Szatmari I, Pap A, Ruhl R, Ma JX, Illarionov PA, Besra GS, et al. PPARgamma controls CD1d expression by turning on retinoic acid synthesis in developing human dendritic cells. J Exp Med 2006; 203:2351–62. [PubMed: 16982809]
47. Villablanca EJ, Wang S, de Calisto J, Gomes DC, Kane MA, Napoli JL, et al. MyD88 and retinoic acid signaling pathways interact to modulate gastrointestinal activities of dendritic cells. Gastroenterology 2011; 141:176–85. [PubMed: 21596042]
48. Zimmer A, Luce S, Gaignier F, Nony E, Naveau M, Biola-Vidamment A, et al. Identification of a new phenotype of tolerogenic human dendritic cells induced by fungal proteases from Aspergillus oryzae. J Immunol 2011; 186:3966–76. [PubMed: 21368225]
49. Correale J, Farez MF. Parasite infections in multiple sclerosis modulate immune responses through a retinoic acid-dependent pathway. J Immunol 2013; 191:3827–37. [PubMed: 23975865]
50. Konieczna P, Groeger D, Ziegler M, Frei R, Ferstl R, Shanahan F, et al. Bifidobacterium infantis 35624 administration induces Foxp3 T regulatory cells in human peripheral blood: potential role for myeloid and plasmacytoid dendritic cells. Gut 2012; 61:354–66. [PubMed: 22052061]
51. Sato T, Kitawaki T, Fujita H, Iwata M, Iyoda T, Inaba K, et al. Human CD1c(+) myeloid dendritic cells acquire a high level of retinoic acid-producing capacity in response to vitamin D(3). J Immunol 2013; 191:3152–60. [PubMed: 23966631]
52. Dorner JW. Management and prevention of mycotoxins in peanuts. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 2008; 25:203–8. [PubMed: 18286410]
53. Ozias-Akins P, Breiteneder H. The functional biology of peanut allergens and possible links to their allergenicity. Allergy 2019; 74:888–98. [PubMed: 30636003]
54. Tordesillas L, Berin MC. Mechanisms of Oral Tolerance. Clin Rev Allergy Immunol 2018; 55:107–17. [PubMed: 29488131]
55. Xi J, Yang Z. Expression of RALDHs (ALDH1As) and CYP26s in human tissues and during the neural differentiation of P19 embryonal carcinoma stem cell. Gene Expr Patterns 2008; 8:438–42. [PubMed: 18502188]
56. Lack G, Fox D, Northstone K, Golding J, Avon Longitudinal Study of P, Children Study T. Factors associated with the development of peanut allergy in childhood. N Engl J Med 2003; 348:977–85. [PubMed: 12637607]
57. Du Toit G, Katz Y, Sasieni P, Mesher D, Maleki SJ, Fisher HR, et al. Early consumption of peanuts in infancy is associated with a low prevalence of peanut allergy. J Allergy Clin Immunol 2008; 122:984–91. [PubMed: 19000582]
58. Du Toit G, Roberts G, Sayre PH, Bahnson HT, Radulovic S, Santos AF, et al. Randomized trial of peanut consumption in infants at risk for peanut allergy. N Engl J Med 2015; 372:803–13. [PubMed: 25705822]
59. Fox AT, Sasieni P, du Toit G, Syed H, Lack G. Household peanut consumption as a risk factor for the development of peanut allergy. J Allergy Clin Immunol 2009; 123:417–23. [PubMed: 19203660]

60. Brough HA, Simpson A, Makinson K, Hankinson J, Brown S, Douiri A, et al. Peanut allergy: effect of environmental peanut exposure in children with filaggrin loss-of-function mutations. J Allergy Clin Immunol 2014; 134:867–75 e1. [PubMed: 25282568]

61. Brough HA, Liu AH, Sicherer S, Makinson K, Douiri A, Brown SJ, et al. Atopic dermatitis increases the effect of exposure to peanut antigen in dust on peanut sensitization and likely peanut allergy. J Allergy Clin Immunol 2015; 135:164–70. [PubMed: 25457149]

62. Tordesillas L, Goswami R, Benede S, Grishina G, Dunkin D, Jarvinen KM, et al. Skin exposure promotes a Th2-dependent sensitization to peanut allergens. J Clin Invest 2014; 124:4965–75. [PubMed: 25295541]

63. Dolence JJ, Kobayashi T, Iijima K, Krempski J, Drake LY, Dent AL, et al. Airway exposure initiates peanut allergy by involving the IL-1 pathway and T follicular helper cells in mice. J Allergy Clin Immunol 2018; 142:1144–58 e8. [PubMed: 29247716]

64. Smeekens JM, Immormino RM, Balogh PA, Randell SH, Kulis MD, Moran TP. Indoor dust acts as an adjuvant to promote sensitization to peanut through the airway. Clin Exp Allergy 2019; 49:1500–11. [PubMed: 31444814]

65. Yoshie O, Matsushima K. CCR4 and its ligands: from bench to bedside. Int Immunol 2015; 27:11–20. [PubMed: 25087232]

66. Hirata H, Yukawa T, Tanaka A, Miyao T, Fukuda T, Fukushima Y, et al. Th2 cell differentiation from naive CD4(+) T cells is enhanced by autocrine CC chemokines in atopic diseases. Clin Exp Allergy 2019; 49:474–83. [PubMed: 30431203]

Key messages:

- Peanut protein signals through TLR2 to induce expression and activity of the retinoic acid-producing enzyme RALDH2 in human myeloid dendritic cells and monocytes.
- Retinoic acid produced by these antigen-presenting cells promotes Th2 differentiation and gut homing in naïve $CD4^+$ T cells.
- These findings shed a new light on the interplay between innate and adaptive immune responses to peanut, and may help explain its potent allergenicity.

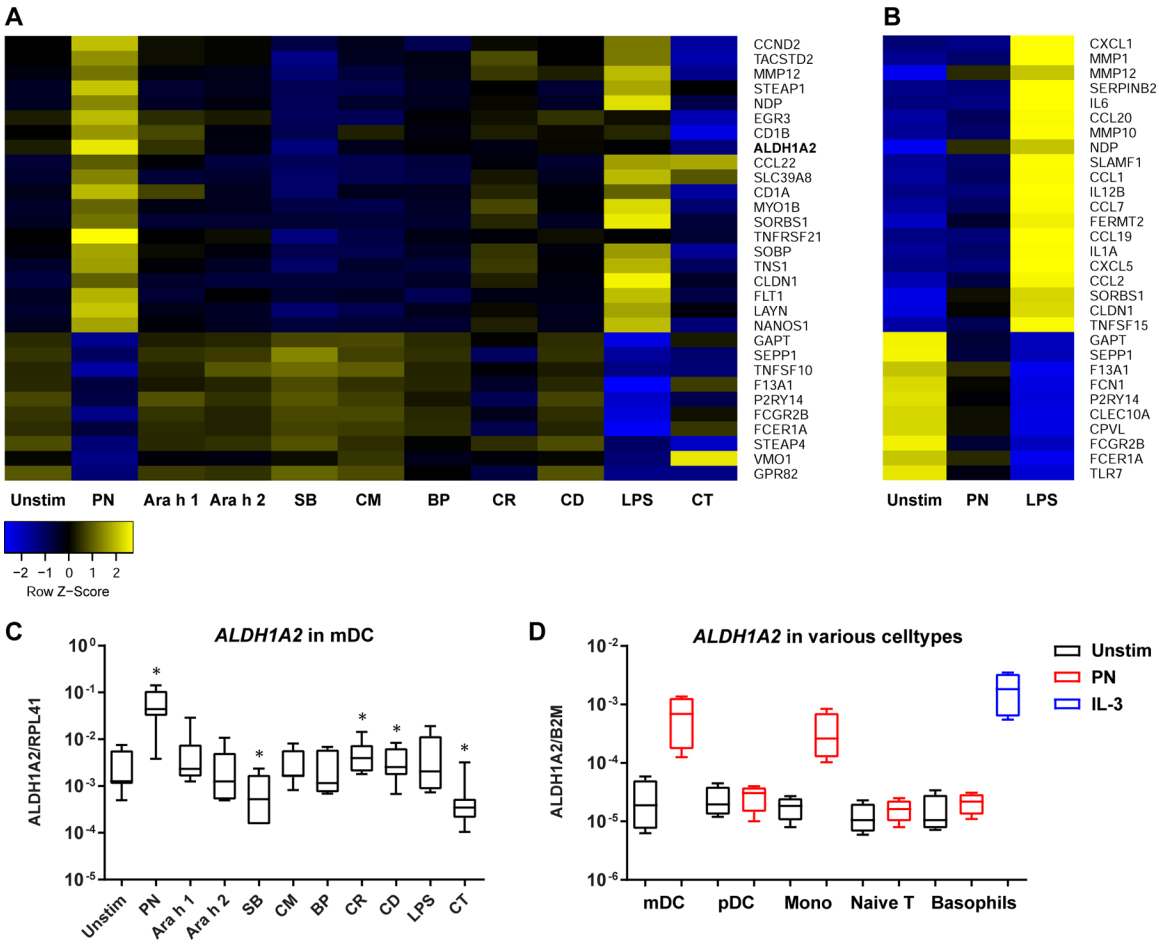


Fig. 1. PN induces a unique gene expression profile in human mDC, including the upregulation of *ALDH1A2*.

A. Heat map showing the top 20 upregulated genes and the top 10 downregulated genes by PN in mDC after 24h incubation, and the induction of these genes by the purified peanut allergens Ara h 1 and Ara h 2, soybean protein (SB), cow's milk protein (CM), birch pollen extract (BP), cockroach allergen extract (CR), cat dander extract (CD), lipopolysaccharide (LPS), and cholera toxin (CT). **B.** Heat map showing the top 20 upregulated genes and the top 10 downregulated genes by LPS, and the induction of these genes by PN. **C.** Expression of *ALDH1A2* relative to housekeeping gene *RPL41* in mDC cultured with the indicated stimuli. Combined data of 7 independent experiments and subjects are shown (* $P < 0.05$; PN/SB/CD vs Unstim: paired *t* test; CR/CT vs Unstim: Wilcoxon matched-pairs signed rank test). **D.** Expression of *ALDH1A2* relative to housekeeping gene *B2M* in unstimulated and PN-stimulated mDC, pDC, monocytes, naïve $CD4^+$ T cells, and basophils. Basophils stimulated with 1 ng/ml IL-3 served as positive control. Combined data of 4 independent experiments and subjects are shown.

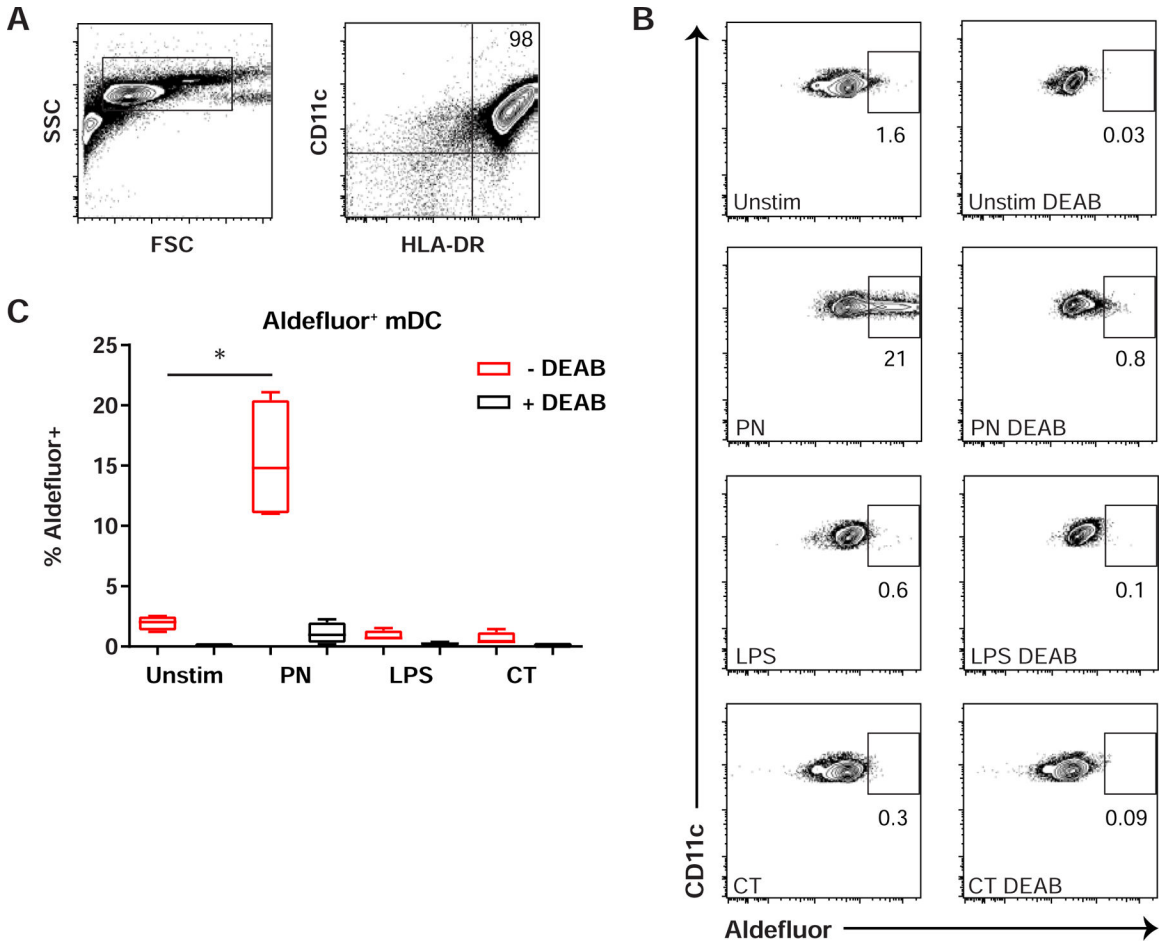


Fig. 2. PN increases enzymatic activity of RALDH2 in mDC.

A. Representative example of the purity of freshly isolated mDC from PBMC. **B.** Representative example of RALDH2 activity in mDC, gated on live HLA-DR^{+}CD11c^{+} cells. The mDC were incubated first with the indicated stimuli, and subsequently with the fluorescent RALDH substrate Aldefluor. The RALDH inhibitor DEAB served as negative control. **C.** Summary of Aldefluor data, obtained from 4 independent experiments and subjects (* $P < 0.05$, paired *t* test).

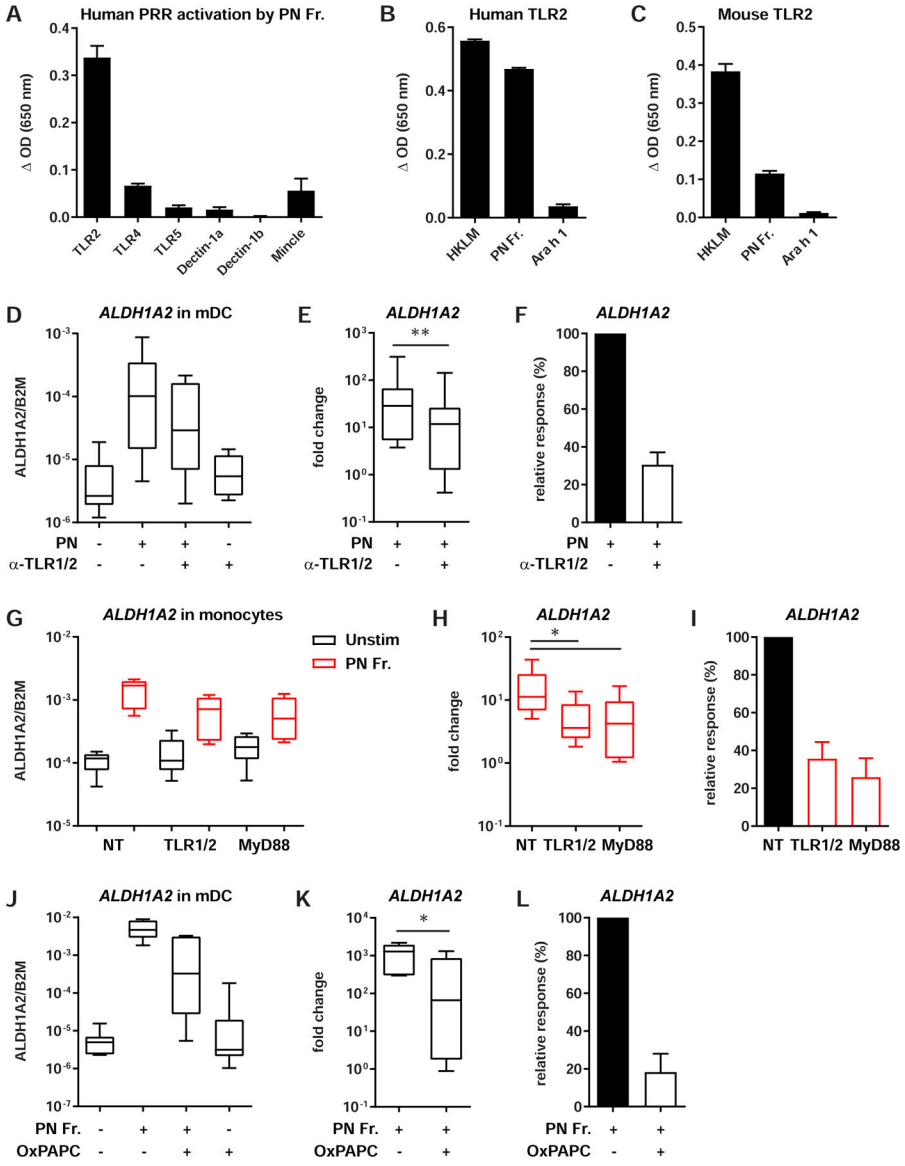


Fig. 3. PN signals through the TLR1/TLR2 heterodimer to induce expression of *ALDH1A2* in mDC and monocytes.

A. HEK293-derived reporter cell lines expressing the indicated human TLRs and CLRs were incubated with PN Fr. in triplicate, and NF-κB-induced expression and activity of secreted embryonic alkaline phosphatase was measured (see Methods). The difference in optical density (Δ OD) between wells with cells incubated with and without PN Fr. is shown. **B, C.** As in panel A, but TLR2 reporter cell lines were incubated with PN Fr. or Ara h 1, or heat-killed *Listeria monocytogenes* (HKLM). **D.** Expression of *ALDH1A2* relative to *B2M* in PN-treated and untreated mDC, incubated with or without blocking anti-TLR1 and anti-TLR2 antibodies. Combined data of 9 independent experiments and subjects are shown. **E.** Fold change in expression of *ALDH1A2* in PN-stimulated mDC treated with or without blocking antibodies (** $P < 0.01$, Wilcoxon matched-pairs signed rank test). **F.** Relative magnitude of PN-induced *ALDH1A2* expression in mDC treated with blocking antibodies as compared to untreated mDC. **G.** Expression of *ALDH1A2* relative to *B2M* in monocytes

treated with non-targeting (NT), TLR1- and TLR2-, or MyD88-specific siRNA, and incubated with or without PN Fr. Combined data of 7 independent experiments and subjects are shown. **H.** Fold change in expression of *ALDH1A2* in PN Fr.-stimulated monocytes treated with the indicated siRNA (* $P < 0.05$, paired *t* test). **I.** Relative magnitude of PN Fr.-induced *ALDH1A2* expression in monocytes treated with TLR1- and TLR2-, or MyD88-specific siRNA, compared to monocytes treated with NT siRNA. **J.** Expression of *ALDH1A2* relative to *B2M* in PN Fr.-treated and untreated mDC, incubated with or without the TLR2 inhibitor OxPAPC. Combined data of 7 independent experiments and subjects are shown. **K.** Fold change in expression of *ALDH1A2* in PN Fr.-stimulated mDC treated with or without OxPAPC (* $P < 0.05$, Wilcoxon matched-pairs signed rank test). **L.** Relative magnitude of PN Fr.-induced *ALDH1A2* expression in mDC treated with OxPAPC compared to untreated mDC.

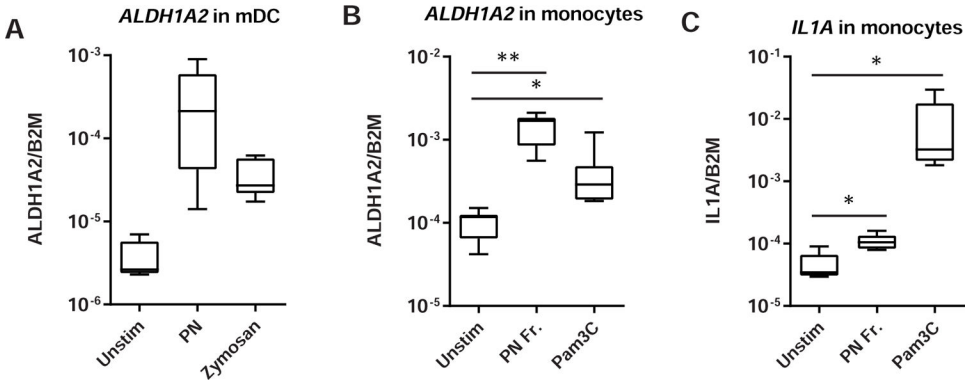


Fig. 4. Zymosan and Pam3CSK4 induce modest expression of *ALDH1A2* in human mDC and monocytes.

A. Expression of *ALDH1A2* relative to *B2M* in mDC treated with or without PN or zymosan. Combined data of 5 independent experiments and subjects are shown. **B.** Expression of *ALDH1A2* relative to *B2M* in monocytes treated with or without PN Fr. or Pam3CSK4. Combined data of 7 independent experiments and subjects are shown (* $P < 0.05$, Wilcoxon matched-pairs signed rank test, ** $P < 0.01$, paired *t* test). **C.** As in panel B, but with *IL1A* as readout gene instead of *ALDH1A2* (* $P < 0.05$, Wilcoxon matched-pairs signed rank test).

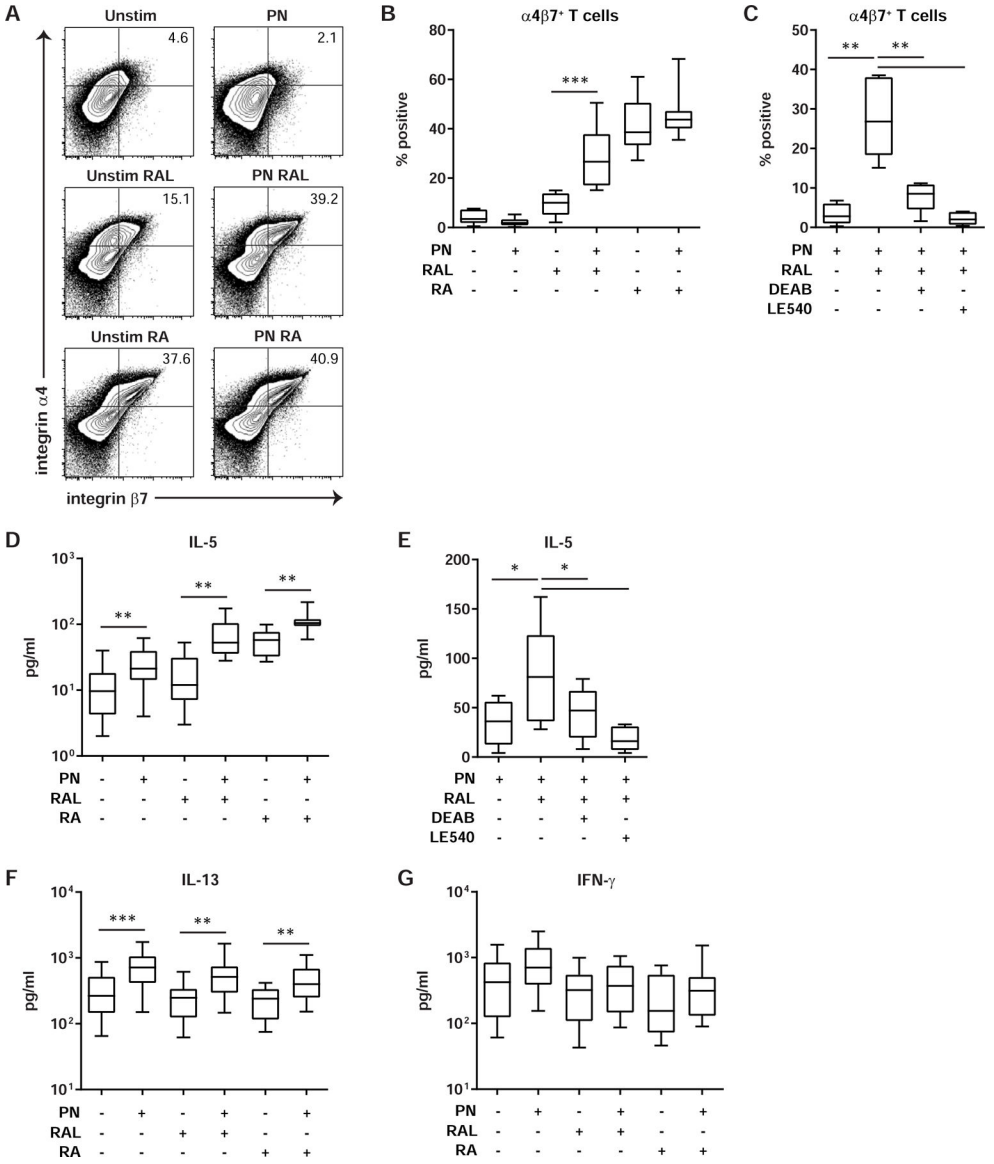


Fig. 5. RA produced by PN-stimulated mDC induces integrin $\alpha_4\beta_7$ and IL-5 in naïve CD4$^+$ T cells.

Naïve Th cells were co-cultured with unstimulated or PN-treated mDC, with or without the RA precursor retinal (RAL) and RA. **A.** Representative example of integrin $\alpha_4\beta_7$ expression on T cells, gated on live CD3$^+$CD4$^+$ cells. **B.** Summary of integrin $\alpha_4\beta_7$ expression data, obtained from 10 independent experiments and subjects (*** $P < 0.001$, paired *t* test). **D, F, G.** Concentrations of IL-5, IL-13, and IFN-γ in supernatants of the co-cultures (** $P < 0.01$, *** $P < 0.001$, paired *t* test and Wilcoxon matched-pairs signed rank test). **C, E.** Frequency of integrin $\alpha_4\beta_7^+$ T cells and concentration of IL-5 in supernatant from co-cultures with PN-treated mDC, with or without RAL, the RALDH inhibitor DEAB, and the RAR antagonist LE540. Combined data of 5 independent experiments and subjects are shown (* $P < 0.05$, ** $P < 0.01$, paired *t* test).