Exposure to Disperse Azo Dyes Promotes Allergic Sensitization to Peanut

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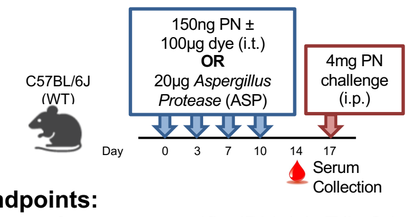
Background

- Environmental exposure to peanut allergen in indoor house dust (ID) through non-oral routes, like airway or skin exposures, may be a risk factor for food sensitization [1,2].
- ID contains various immunostimulatory agents, including pollutants and synthetic chemicals [3].
- Recently, a class of textile dyes known as disperse azo dyes was found in ID [4].
- Disperse azo dyes are known immune sensitizers, but it is unknown if they act as adjuvants and promote allergic sensitization to foods.

Objective

- **Investigate if disperse azo dyes can act as adjuvants and promote peanut sensitization and allergy development using human cell line and mouse models.**

Airway Sensitization Model



Primary Endpoints:
- Serum levels of peanut-specific (PNs) IgE/IgG1
- Anaphylaxis (i.e., decreased body temperature) after intraperitoneal (i.p.) PN challenge

Statistical Analysis:
- Data are shown as mean ± SEM. Differences between groups were determined by t-tests, one- or two-way ANOVA, and a p-value <0.05 was considered significant. *p<0.05, **p<.0.01, ***p<0.001, ns, non-significant.

Conclusions

- Allergens like peanut and immune adjuvants including several disperse azo dyes are present in ID
- Airway co-exposure to Disperse Orange 25 (DO-25) and peanut can induce allergic sensitization to peanut.
- IL-1β signaling is required for DO-25 mediated allergic sensitization to peanut.
- This research has been submitted to a peer-reviewed journal.

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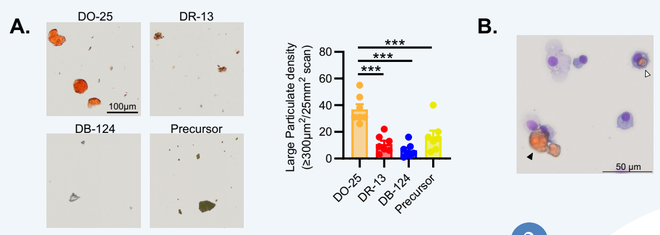
References
1. Smeekens et al. Clin Exp Allergy. 2019;49(11):1500-1511
2. Kulis et al. J Allergy Clin Immunol. 2021;148(3):689-693.
3. Moran. Curr Allergy Asthma Rep. 2020;20(8):37.
4. Overdahl et al. Environ Pollut. 2023;337:122491

Results

DO-25 forms particulates in aqueous solution. (A) Microscopic analysis of disperse azo dye particulate. **(A, Left)** Brightfield images of 100 µg/mL disperse azo dyes. **(A, Right)** Density of particulates ≥300 µm² per 5 mm x 5 mm scan field. **(B)** Brightfield image of BAL cells collected at 24 hours post instillation of PN with DO-25 stained with Kwik-Diff. Small DO-25 particulate could be phagocytosed (**white arrow**), while large DO-25 particulate was surrounded by alveolar macrophages but not phagocytosed (**black arrow**).



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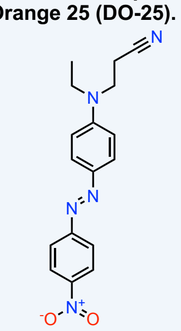
Structure of Disperse Orange 25 (DO-25).



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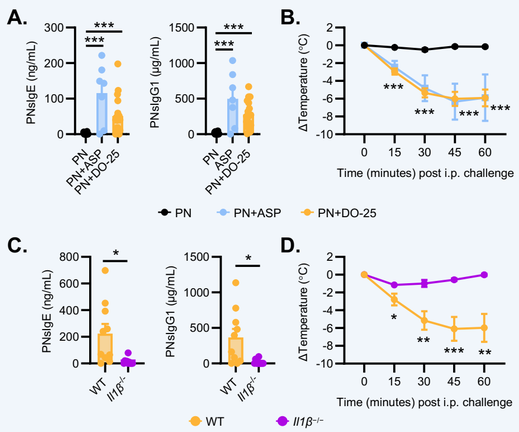

Airway exposure to disperse azo dyes induces IL-1β-dependent peanut allergy in mice. (A and C) PNsIgE or PNsIgG1 measured following airway sensitization. **(B and D)** Change in core body temperature following i.p. peanut challenge. **(A-B)** Experiments were performed with wildtype (WT) mice. **(C-D)** Experiments were performed to compare WT to IL-1β$^{-/-}$ mice.

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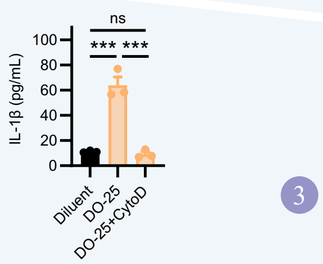

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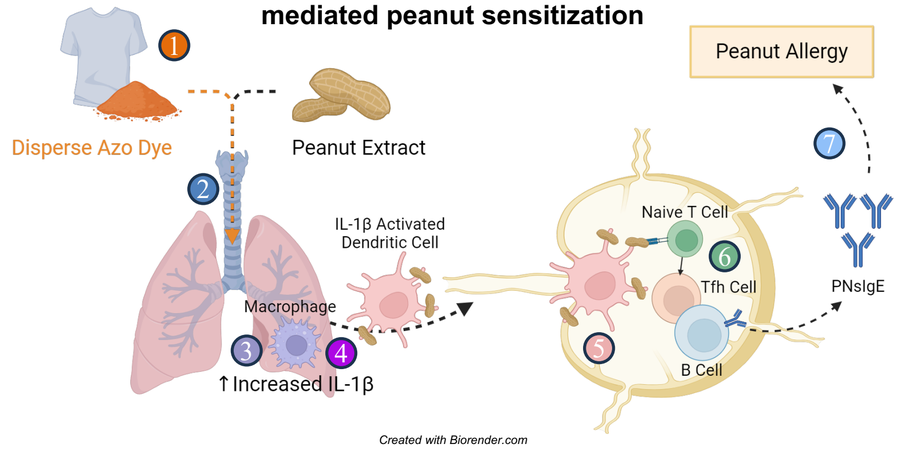
DO-25 particulates drive phagocytosis-dependent IL-1β production by human THP-1 macrophages. Production of IL-1β following treatment with DO-25 was compared to the diluent control and DO-25 plus cytochalasin D (CytoD). Treatment with CytoD prevents phagocytosis.

Proposed model of DO-25-mediated peanut sensitization



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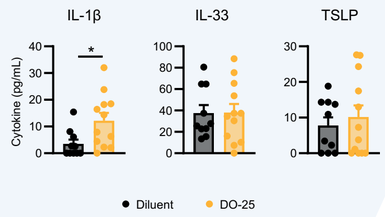

DO-25 exposure leads to IL-1β activation. Innate cytokines were measured in BALF collected 4 hours after treating mice with 100 µg DO-25 or vehicle control 2% (v/v) DMSO (Diluent).

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Inhaled DO-25 induces maturation of migratory lung conventional dendritic cells. Mice were exposed to 100 µg DO-25 or Diluent. After 24 hours, mediastinal lymph nodes (mLN) were collected for analysis by flow cytometry. **(A)** Gating strategy for the identification of migratory cDC1 and cDC2 cells. **(B)** Number of migratory or resident cDCs. **(C)** Median fluorescence intensity (MFI) of the CD80/86 (maturation markers) fold-change compared to Diluent.

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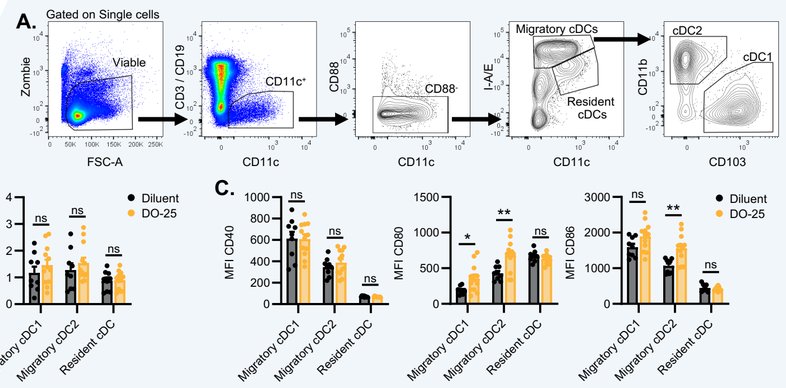

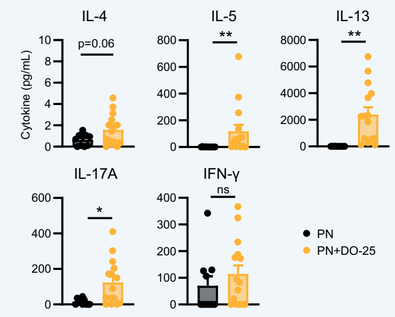

DO-25 promotes peanut-specific Th2 and Th17 responses. T-helper cytokines were measured in lung-draining mLNs collected on day 14 after treating mice with 150 ng PN protein alone, 100 µg DO-25, or PN and DO-25. mLNs were restimulated *ex vivo* with 100 µg/mL peanut protein. Th cytokines levels in mLN cell culture supernatants were measured following 4 days of culture.