Augmentation of Oral Immunotherapy with Tolerance-induce Nanoparticles

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Statement of Purpose: Roughly 15 million Americans suffer from food allergies which, in severe cases, can be life threatening. Allergic individuals typically manage their conditions through strict food avoidance and/or the adminstration of antihistamine upon accidental exposure[1]. Presently, oral immunotherapy (OIT) is the most efficacious option to achieve sustained unresponsiveness (SU) in allergic paitents but is limited by the risk of triggering anaphylaxis and requiring intensive medical supervision[2]. Several clinical studies indicate that SU after OIT is correlated with increased T regulatory cell populations, which suggests the supplementation of OIT with tolerogenic immunomodulatory factors will increase its efficacy[3,4,5]. Polysaccharide A (PSA), a commensal molecule produced by the gut-symbiont *Bacterorides fragilis*, has been shown to have Treg-inducing capabilities within the gut[6]. The tolerance-inducing capacity in combination with its polymeric structure makes PSA an intriguing biomaterial for the formulation of tolerogenic nanoparticles. We hypothesize that the encapsulation of allergen within PSA nanoparticles (NPs) will show significant improvements in efficacy and safety over traditional allergen-only oral immunotherapy due to PSA NPs ability to (i) induce Treg differentiation and deliver allergen simultaneously, and (ii) to shield the allergen from IgE receptor-mediated mast cell activation until internalization by intestinal dendritic cells **(Figure 1)**. We show that PSA NPs can be readily fabricated and maintains its ability to stimulate TLR2.

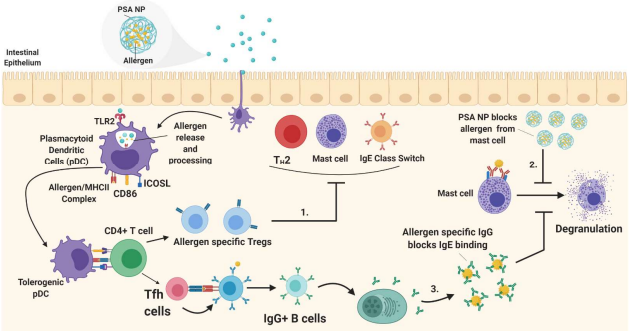

Fig 1: Proposed mechanism of oral tolerance induction by allergen-encapsulated-PSA NPs (1.) PSA NP mediates tolerogenic pDC differentiation and particle uptake and presentation of allergen-derived peptides stimulates the expansion of allergen-specific Tregs (2.) PSA NPs sequester allergen from specific IgE on mast cells and reduces likelihood of allergic responses (3.) Allergen-encapsulated-PSA-NPs could induce blocking IgG antibodies that prevent allergen/IgE interactions and mast cell degranulation.

Materials and Methods: Water soluble molecules were isolated from PSA-only expressing *B. fragilis* (provided by L. Comstock, Harvard) via phenol/chloroform-water extraction. Extracts were treated with RNase, DNase, and pronase prior to size exclusion chromatography (SEC). Fractions were analyzed by SDS-PAGE/Emerald 300 staining. PSA purity and structure was confirmed by H^1 NMR (Bruker 800). PSA NPs were fabricated using water/oil emulsification with chemical crosslinking. Morphology and size were analyzed with scanning electron microscopy (SEM) and dynamic light scattering (DLS). HEK-Blue mTLR2 reporter cells (Invivogen) were treated with 25ug/ml PSA, PSA NPs, ovalbumin (OVA)-loaded PSA-NPs, soluble PSA plus OVA, LPS, or purified water for 16 hours in the absence or presence of blocking anti-mTLR2 antibody. Loaded particles were fabricated with either 30:1 or 3:1 PSA to OVA to PSA mass ratios. Subsequent activity was measured by absorbance at 625nm and normalized to water. The same sample groups were used as treatments in a mixed lymphocyte reaction (MLR) assay consisting of dendritic and OVA-specific T cells derived from OT-II mice. IL-10 production was measured by ELISA after four days of incubation.

Results: SEM and DLS reveal the spheroidal morphology of PSA NPs with an average size of 200nm **(Figure 2A, B)**. PSA-NPs and OVA-loaded PSA-NPS showed slightly lower specific mTLR2 specific activity than unaltered PSA, but significantly higher activity than both LPS and water controls. OVA-loaded PSA nanoparticles and soluble PSA plus OVA co-incubation induced IL-10 production in OVA-specific MLR **(Figure 2C, D)**.

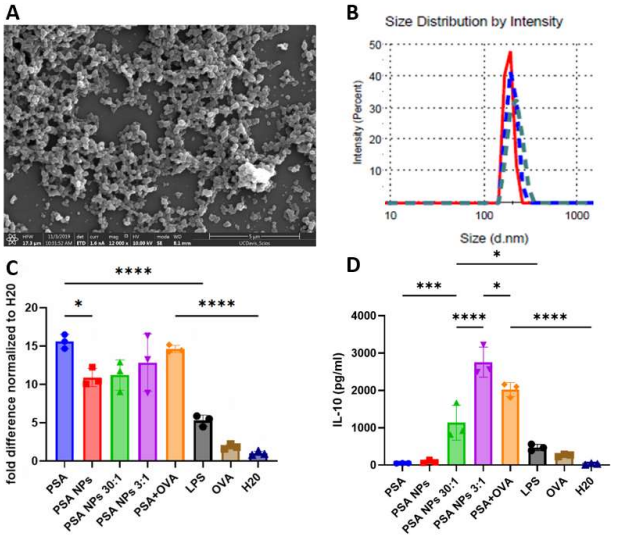

Fig 2: PSA NP morphology, size, and functionality. (A) SEM of and B) DLS of PSA-NPs indicates the average particle size of 200nm. (C) Specific mTLR2 activity determined via HEK-Blue mTLR2 reporter cell assay. (D) IL-10 production after OVA-specific mixed lymphocyte reaction

Conclusion: Our results indicate that PSA NPs can be fabricated using a water/oil emulsification strategy and be loaded with OVA protein while maintaining PSA's immunomodulatory capacity. Moreover, IL-10 production after treatment with OVA-loaded PSA-NPs suggests that PSA-NPs can deliver protein for MHC processing while inducing tolerogenic responses. Complete characterization of dendritic and T cells will be elucidated in future experiments. Furthermore, allergen-loaded PSA nanoparticles will be tested in food-induced allergy mouse models to evaluate their ability to augment OIT.

References: 1. Wong L et al., Curr Opin Allergy Clin Immunol. 2019 Jun;19(3):236-242 2. Wood RA. J Allergy Clin Immunol. 2016;137(4):973–982. 3. Pellerin L et al., J Allergy Clin Immunol. 2018 Jan;141(1):202-213.e8. 4. Tordesillas L et al., J Allergy Clin Immunol. 2017 Jan;139(1):189-201.e4. 5. Burton OT et al., Immunity. 2014 Jul 17;41(1):141-51. 6. Erturk-Hasdemir D, Kasper DL.. Ann N Y Acad Sci. 2018 Apr;1417(1):116-129. 7. Tzianabos AO et al., J Biol Chem. 1992 Sep5;267(25):18230-5.