Purpose: Report induction of tolerance to poison ivy urushiol by precipitation of insoluble allergen in muscle & initial studies to adapt the same method to Ara h2 for peanut allergy.

Poison Ivy (PI): Background: At AAAAI 2010 we reported induction of tolerance in 2 patients (pts) highly allergic to PI with less than 1 mg urushiol administered intramuscularly in small volumes of 95-100% ethanol. Quantitative patch test sensitivity mirrored clinical response.

Methods:

Vaccines: 
- **Pi1**: 7 day crude ethanol extract of fresh poison ivy leaves, urushiol content ~2 mg/ml, limited long term stability.
- **Pi2**: Purified (88% of total mass), stable solution of urushiol extracted from fresh leaves, dissolved in 100% ethanol @ conc 50 mg/ml.
- **Pi3**: Mix of 25% by volume of **Pi2**: 75% fresh **Pi4**.
- **Pi4**: 7 day crude ethanol extract evaporated to dryness, re-dissolved in 100% ethanol @ conc 50 mg/ml.

Urushiol assay: Lots of Pi1 made in 2008 and 2009 were assayed by gas chromatography and mass spectrometry by Dr. Richard Sicher of the USDA-ARS Plant Sciences Institute. Pi2, Pi3 and Pi4 were assayed at Rowan University by the same method.

Urushiol congener distribution:
Poison ivy urushiol exists in saturated, mono, di and tri-unsaturated congeners in nature. Allergenicity increases with degree of unsaturation. The distribution of congeners measured by gas chromatography / mass spectrometry was similar in Pi1, Pi2, Pi3 and Pi4.

Quantitative Patch Test: The method of Marks was adapted (table 1) with multipliers to calculate equivalent “grade 3” dose estimated from dose-ranging and clinical observation.

Patch test technique and examples of reactions are shown in Fig’s 1 & 2.

Clinical trial protocol: After informed consent pts c/ difficult-to-avoid recurrent severe PI were patch-tested, treated (Tx) by series of IM injections c/ concurrent H1 antihistamine, monitored for toxicity by Sx, CBC, UA, multi-chem and re-tested at intervals after completing Tx.

Results:
- Patients (Pts) who completed testing & treatment are listed in table 2. Every Pt followed serially had constant “non-tolerant” baseline tests.
- Pi1: 7-day crude ethanol extract of fresh leaves with ~2 mg/ml urushiol. Pi2: 75% fresh, Pi3: 25% Pi2, Pi4: 7 day crude ethanol extract.

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our 4th generation vaccine (Pi4) has the safety, efficacy, reproducibility and stability necessary to meet FDA requirements for commercial licensure and that publication should help us find an industry partner for commercial development.

**Peanut (PN):**

**Background:** The same mechanism of T-regulatory cell tolerogenesis is common to humoral and cell mediated immunity. A vaccine delivery system that successfully induced tolerance to poison ivy may be similarly effective in humoral allergy. Conversion of protein allergens into allergoids by cross-linking or other means increases their ratio of tolerogenicity to allergenicity, presumably by reducing access by cell-bound IgE molecules to epitopes able to trigger reactions. Allergoids act to expand the T-regulatory cell population in competition with Th1 and Th2 enhancement of allergic sensitization. As our vaccine delivery system appears to enhance T-cell population shift in the same pro-tolerogenic direction it is possible that the two interventions in combination may be synergistic, i.e., more effective than simply additive.

We propose to make allergoids of Ara h2 that are soluble in ethanol but insoluble in water, and study the same vaccine delivery system in animal models of peanut allergy.

**Methods:** We propose to make allergoids by four methods; 1) Cross-linking cysteines with glutaraldehyde; 2) Cross-linking cysteines with succinic acid; 3) Carbamylation of lysine residues with potassium cyanate and 4) Thiol-esterification of cysteines with monovalent aldehydes such as formaldehyde and valeraldehyde. There are other known side-chain coupling reactions to modify protein solubility and we have also designed novel side chain coupling reagents to further reduce solubility in water and increase it in ethanol. As all of these reactions involve coupling to reactive amino acid residues, it is possible that variation in the choice of cross-linking agent, time and conditions of coupling reaction, molar ratio of cross-linking agent to Ara h2 and choice and reaction conditions for solubility-modifying side-chain coupling will differentially affect the tolerogenicity of the resulting materials when used as allergy vaccines. We will therefore make differently formulated allergoids that form low viscosity solutions in ethanol and are insoluble in water.

**Preliminary results:** We made small batches of glutaralergoid varying the glutaraldehyde (glut) : Ara h2 molar ratio from 4 to 10 and valeralergoids over a 3-fold range of valeraldehyde : Ara h2. A molecular sizing gel is shown in Fig. 3. The glutaralergoids formed at higher glut : Ara h2 ratios show reduced solubility in water. We have not yet purified sufficient quantities of valeralergoids to study their solubility.

**Follow-up:** We plan to increase both cross-linking of and hydrophobic binding to Ara h2 until we obtain allergoids that are insoluble in water, and then make them soluble in ethanol by N-glycosylation. Dr. Robert Hamilton will test the resulting allergoid vaccines for lack of allergenicity (low or zero binding with IgE from peanut-allergic patients) and Drs. Angela Haczku of the University of Pennsylvania and Kingsley Yin of Rowan University will evaluate them for tolerogenesity in small animal models of peanut allergy.

**References:**


