611 Dose-Dependent Anti-inflammatory Effect of Inhaled Mometasone Furoate/Formoterol in Subjects With Asthma and High Baseline Exhaled Nitric Oxide and Sputum Eosinophils

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RATIONALE: We investigated dose-responses of inflammatory markers (fractional exhaled nitric oxide [FeNO], sputum eosinophils) to inhaled mometasone furoate/formoterol (MF/F) administered via metered-dose inhaler (MDI) in asthmatics.

METHODS: Ninety-two atopic adult asthmatics (baseline FeNO 55-103ppb; sputum eosinophil levels >13%) completed a randomized, double-blind, parallel-group study; 55% previously used ICS (fluticasone equivalent=110-1000μg/d) with/without long-acting β_2-agonists. After a 2-wk ICS washout, subjects received twice-daily MF/F-100/10μg, MF/F-200/10μg, MF/F-400/10μg, MF-MDI-200μg, MF-dry powder inhaler (DPI)-200μg, or placebo for 2wks. Change from baseline-day 14 in mean % change FeNO (primary outcome) was analyzed using ANCOVA with baseline FeNO as a covariate; median changes in sputum eosinophils % and mean changes in morning predose peak expiratory flow (PEF) were secondary outcomes.

RESULTS: A dose-response effect on FeNO at endpoint occurred across escalating doses of MF/F (1.61, 1.86, 2.49 mean-fold differences from placebo, respectively; trend test, $P<0.001$). For % changes in FeNO at endpoint, all MF/F groups were significantly superior vs placebo (-47% to -66% vs -15%; $P\leq0.012$), with ICS treatment effects occurring as early as day 7. An MF/F dose-response trend in reduction of median eosinophils % was also observed ($P\leq0.065$). PEF improvements with MF/F (10.3%-16.6%; no dose-response trend) were significantly superior to PEF reduction with placebo (-1.7%; $P\leq0.002$); MF/F-400/10μg was significantly superior to MF-DPI-200μg and MF-MDI-200μg ($P\leq0.034$).

CONCLUSION: Inflammatory markers show an MF/F dose-dependent reduction within 14 days in subjects with high baseline FeNO and sputum eosinophils (presumably due to MF). These measurements are more sensitive and require smaller sample sizes than PEF for evaluating anti-inflammatory treatment pharmacodynamics of MF/F.

612 Single-Species Grass Pollen Extracts and Mixtures of Extracts From Multiple Phylogenetically Related Grass Species Activate Allergic Patients' Basophils to the Same Degree

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RATIONALE: The necessity of complete identity between the sensitizing allergens and allergens used for immunotherapy has been debated. We have previously demonstrated that the effect of individual Derp2 isoforms on basophil activation is minimal, given a polyclonal IgE response (Christensen et al Journal of Immunology (2010) 184, 4966). Similarly, we here demonstrate that extracts of single-pollen and mixes of multiple grass species, respectively, activate basophils from grass-pollen allergic individuals to the same extent.

METHODS: Blood basophils from grass allergic donors or basophils sensitized with IgE from grass allergic donors were incubated with either *Phleum pratense*, a 5-grass (*Dac glo, Fes pra, Lol per, Phl pra, Sec cer*) or 7-grass mix (*Dac glo, Fes pra, Lol per, Phl pra, Poa pra, Arr ela, Sec cer*). Activation was measured by flow cytometry. Also, alum-formulated vaccines in the form of *Phleum pratense* alone or grass mixes were compared in basophil activation assays.

RESULTS: No differences in the ability of the single-species *Phleum pratense* extract and the 5- or 7-grass mixes to activate basophils from individual allergic donors were seen. Comparable responses were maintained after formulation of *Phleum pratense* and 5- and 7- species grass mixes with ALOH.

CONCLUSIONS: Identical responses to single-species and multiple-species extract mixes in basophil activation assays indicate that in allergic individuals antibody responses to epitopes that are shared between different related grass species dominate over species-specific epitopes and -isoforms. This is in agreement with the confirmed clinically efficacy of single-species (*Phleum pratense*) grass pollen vaccines, both in subcutaneous and Allergy Immunotherapy Tablet regimens.

613 Jug r 1 and Jug r 2-specific CD4+ T-Cells: Epitope Mapping, Ex Vivo Characterization, and Phenotype Analysis

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RATIONALE: Walnut is a common cause of tree nut allergy. Jug r 1 and Jug r 2 are important allergenic English walnut proteins. Identifying allergic CD4+ T-cell epitopes in Jug r 1/Jug r 2 and analyzing the phenotype of walnut-specific CD4+ T-cells could clarify the mechanism behind walnut allergy.

METHODS: Peripheral blood mononuclear cells from walnut-allergic subjects were isolated, stimulated with Jug r 1/ Jug r 2 peptides, and cultured for 2 weeks. Tetramer-guided epitope mapping (TGEM) was performed with MHC class II tetramers to identify HLA-restricted CD4+ T-cell epitopes using flow cytometry. The tetramer-positive cells were enriched using anti-phycoerythrin magnetic beads to determine frequency and phenotype. Tetramer-positive cells were cloned to evaluate cell-surface markers and intracellular cytokine profile.

RESULTS: TGEM identified multiple Jug r 2 epitopes specific to HLA DRB1*0101, *0404, *0701, *1401, and *1502, and one Jug r 1 epitope specific to DRB1*0404. Similar responses were absent in non-allergic controls. The frequency of walnut-specific CD4+ T-cells ranges from 1:170,000 to 1:400,000, and these cells lack CRTH2 expression. Cell surface marker and intracellular cytokine staining in clones are consistent with TH2 phenotype.

CONCLUSIONS: Jug r 1 and Jug r 2-specific CD4+ T-cells are present in walnut-allergic subjects and absent in non-allergic subjects, suggesting Jug r 1 and Jug r 2 likely play a role in walnut allergy. Prolonged walnut avoidance likely led to low frequencies of allergen-specific T-cells and lack of CRTH2 expression, whereas clones of tetramer-positive cells exhibit a TH2 phenotype due to stimulation with walnut peptides.

614 Broad Aberration In The Humoral Immune Responses To An Aeroallergen

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RATIONALE: Mountain cedar sensitive patient produce IgE antibodies (Abs) to both conformational and linear epitopes of the dominant allergen, Jun a 1. We compared the serum IgE, IgG and IgA responses to linear and conformational epitopes to asses the structural forms that induce these responses.

METHODS: Two groups of mountain cedar-exposed, atopic adults: cedar skin-prick test positive (SPT+ n=12) and negative (n=8) groups were compared. Native Jun a 1 was denatured by exposure to 6M guanidine. We compared the reactivity of serum IgE, IgG and IgA to native (conformational + linear) and denatured (linear + cryptic) epitopes of Jun a 1, using ELISA. The results are expressed as mean±SD of the %change in reactivity after denaturation of Jun a 1.

RESULTS: In the SPT+ group, IgE reactivity decreased (53±23) while IgA, IgG and IgG4 increased (30±46, 39±38 and 6±56%), ($p<0.0005$, 0.0005 and 0.01) respectively, indicating that IgE recognizes predominantly conformational epitopes and IgA and IgG recognize linear epitopes of Jun a 1. In the SPT - negative group, IgE was detectable in only one subject and the %change in their IgA: 131±131 and IgG: 81±53, were significantly greater than for SPT+, $p<0.05$, indicating IgG and IgA from SPT - group recognize predominantly cryptic epitopes.

CONCLUSIONS: IgE from mountain cedar allergic donors recognize predominantly conformational epitopes, while their IgA and IgG Ab recognize surface-exposed linear epitopes. IgA and IgG from SPT - donors recognize cryptic epitopes. This pattern of epitope recognition indicates broad aberrations in the humoral immune responses to sensitizing allergens.

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