SATURDAY

221 The Role of Adjuvant 1.25-Dihidroxyvitamin D3 on $CD4^+CD25^+CD127^{low}$ Regulatory T-cell in Subcutaneous Allergen Specific Immunotherapy

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RATIONALE: 1,25 Dihydroxyvitamin D3, the active form of vitamin D has been reported to inhibit proliferation of effector cells and to enhance $CD4^+CD25^+CD127^{low}$ regulatory T-cells. We hypothesized that using the active form of vitamin D as an adjuvant would enhanced $CD4^+CD25^+CD127^{low}$regulatory T-cells as well as the anti-inflammatory cytokines of allergic rhinitis patient undergoing subcutaneous immunotherapy (SCIT).

METHODS: It was a randomized, double-blind, placebo-controlled trial conducted in 36 allergic rhinitis patients who suffer vitamin D deficiency; age 18-49 years. The intervention based on patient treated with SCIT–calcitriol 0.5 mcg/day and SCIT–placebo as the control group on immunotherapy build-up phase. All groups evaluated at baseline, week 8 and week 15 for proportion $CD4^+CD25^+CD127^{low}$ regulatory T-cells using flow cytometry, IL-10, and TGF-β using ELISA. Nasal symptoms score using Visual Analoq Scale. The Mann Whitney test, Friedman test and post-hoc test using Wilcoxon used for statistical analysis.

RESULTS: The proportions of $CD4^+CD25^+CD127^{low}$ regulatory T-cells significantly increased in the intervention group compared with control ($p=0.04$) in week 8. There was a significant and positive tren in the increase of anti -inflammatory cytokines in the intervention group at week 8, particulary in IL-10 ($p=0.035$) and TGF-β ($p=0.001$), whereas control groups show after the week 15. The decrease in nasal symptoms score did not differ significantly between groups ($p>0.05$).

CONCLUSIONS: Immunologic outcomes changes start in week 8 in SCIT with 1.25-Dihidroxyvitamin D3 group. However, clinical improvement was mostly similar between groups. 1.25-Dihidroxyvitamin D3 a promising adjuvant for the improvement of SCIT.

222 Effects of topical in vivo treatment on ovalbumin antibody production in mice

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RATIONALE: Management of allergic rhinitis includes avoiding the exposure of allergens combined with drug therapy. For the patients that do not have substantial relief in allergic rhinitis symptoms or incur side effects, subcutaneous and sublingual immunotherapy can be a second line of treatment. This study looked at an alternative topical route of immunotherapy administration of allergen through the skin which may be beneficial in treating allergic rhinitis. IgE, IgG and lymph node T cell responses in mice were studied using ovalbumin antigen mixed with topical cream formulations with potential adjuvant properties applied to the skin.

METHODS: BALB/c mice were immunized with ovalbumin mixed with alum to induce an IgE response. After 14 days ovalbumin mixes with topical creams and controls were applied to the back skin of the mice and IgE, IgG2a and T Cell biomarkers from draining lymph nodes were measured up to 71 days and results compared with intraperitoneal injection controls.

RESULTS: Mice developed IgE to ovalbumin after 14 days and application of cream formulations showed a dose dependent reduction in IgE compared to controls at 42 and 71 days. Topically applied ovalbumin showed an increase in sIgG2 comparable to intraperitoneal injection in some formulations and biomarkers such as CD69 indicated that the antigen reached draining lymph nodes.

CONCLUSIONS: Initial results from this mouse study demonstrated that topical administration of allergens mixed in creams can have an immune response and may provide an alternative route for immunotherapy.

223 Experimental Allergen Immunotherapy with Monomeric Allergoid From House Dust Mite *Dermatophagoides pteronyssinus* in a Mouse Model of Allergic Rhinitis

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RATIONALE: Allergen immunotherapy (AIT) with monomeric succinylated allergoid (sD1) of *Dermatophagoides pteronyssinus* (D1) was assessed in a mouse model of allergic rhinitis (MAR).

METHODS: BALB/c mice were immunized with non-modified extract D1 (50 μg/mouse) with aluminum hydroxide (2 mg/mouse) 3 times at 3 week intervals and 6 weeks after challenged by intranasal 50 μl/mouse of D1. AIT occurred between immunization and the beginning of challenge: Group 1 "sham AIT" with 16 SQ injections of PBS; Group 2 16 SQ injections of non-modified D1 in increasing doses; Group 3 8 SQ injections of sD1 in increasing doses; Group 4 combined AIT of 4 SQ injections of sD1in doses of 100, 550, 1000 μg/mouse and 4 sublingual (SL) administrations of sD1 1000 μg/mouse; Group 5 negative control with sham immunization, AIT and PBS challenge. Sneezing (cpm) and breath frequency (plethysmography) were evaluated after challenge with animals sacrificed for histology. Levels of anti-Der p IgE, IgG1, IgG2a in sera were determined by ELISA.

RESULTS: Groups 2, 3, and 4 had significantly reduced sneezes, especially Group 3. Breath frequency was greater in Groups 2 and 4 then Group 1. Anti-Der p IgE in Groups 1, 2, 3 and 4 was elevated versus Group 5. Anti-Der p IgG1 was significantly increased in Groups 2, 3, 4 during AIT and after challenge. Histology showed complete suppression of inflammation in Groups 3 and 4.

CONCLUSIONS: AIT with monomeric succinylated allergoid from house dust mite was effective, with combined subcutaneous and sublingual AIT being most effective.