Th17-derived Cytokines Might be Involved in the Allergic Contact Dermatitis Induced by Poison Ivy Active Ingredient, Urushiol

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1 INTRODUCTION

Poison ivy-induced allergic contact dermatitis (ACD) is a major public health issue and our current understanding of the mechanisms of poison ivy-induced ACD is still poor. Urushiol, the active ingredient in poison ivy, is known to mediate the ACD. However, there are currently no robust preclinical animal models available to assess the pharmacology and mechanistic aspects of poison ivy ACD. In the present experiment, we established a protocol in mice for reproducible induction of dermatitis by the poison ivy active ingredient, urushiol. Using flow cytometry and qRT-PCR, we also identified that Th17-derived cytokines may underlie poison ivy-induced skin reactions.

2 METHODS

Animal protocol: Animals were first sensitized with urushiol by application on shaved abdominal skin on Day 0. Afterwards, animals received several challenges with urushiol from Day 9 by application on both ears. Starting from Day 9, thickness of the ears was measured with a micrometer and redness was scored visually on scale of 0-4 (0-none, 1- very mild, 2-mild, 3-moderate, 4-severe).

qRT-PCR: Total RNA was extracted from mouse ear tissue, and RNA quantity and quality was further assessed. Expression of il17a, il17f, il22 and of gapdh as a housekeeping gene was assessed by quantitative PCR, using a Taqman assay and a One-Step Superscript™ qRT-PCR Kit. Data was reported as the relative fold change for each gene of interest in relation to the housekeeping gene, calculated based on the ΔΔCt method

Flow cytometry: Ear tissues were processed in single cells suspension by enzymatic treatment. Cells were then stimulated using PMA and Ionomycin in the presence of Brefeldin A and incubated overnight. After incubation, cells were harvested and stained using appropriate antibodies targeting Th1, Th17 and Tregs populations and followed by flow cytometry analysis (only Th17 data are shown).

3 RESULTS

Optimization of sensitization and challenge protocol

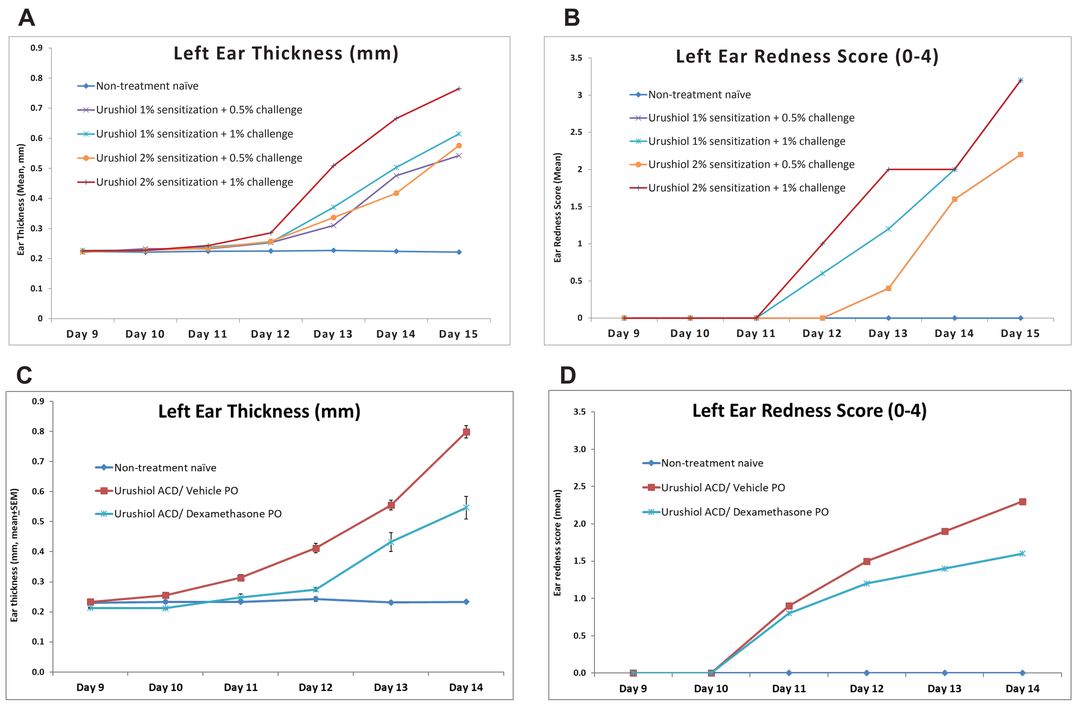

Figure 1: A, B) Following a sensitization with 1% or 2% urushiol on **Day 0 on abdominal skin**, repeat **challenges on the ear** with 0.5% or 1% urushiol were performed on Days 9, 11, 12, 13 and 14. Ear thickness and redness were measured on Days 9, 10, 11, 11, 13, 14 and 15. Results suggest that 2% sensitization with 1% challenge of urushiol induced maximal disease and thus was used for follow up studies with test agents. C, D) Treatment of urushiol ACD mice with steroidal drug dexamethasone from Day 9 reduced the disease levels. N=3-5 per group. Similar changes were also observed in the right ear (data not shown).

3 RESULTS continued…

Flow cytometry in the ear tissues

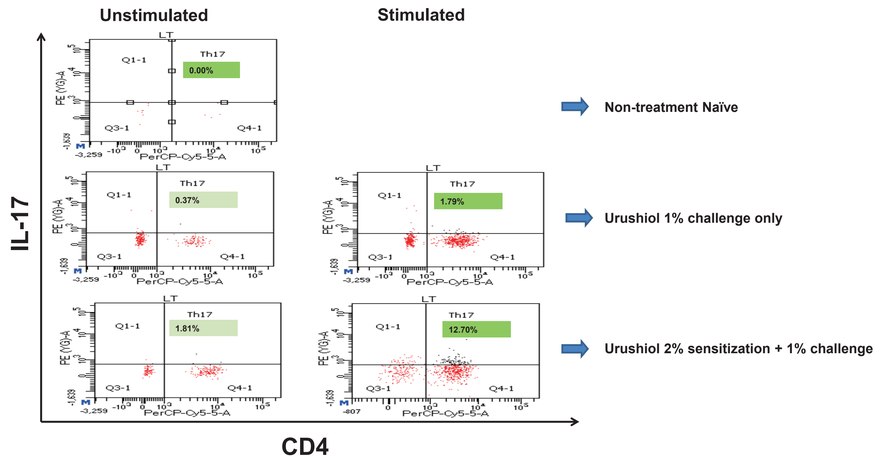

Figure 2: Flow cytometry analysis for profiling of T cell subtypes were performed in the ear tissues collected on Day 15. Only Th-17 data are shown here. There were no Th17 cells present in the ear from naïve mice. However, following urushiol application, Th17 cells were infiltrated into the ear tissues of mice that received multiple challenges without sensitization as well as in animals that received both challenges and sensitization. Extent of infiltration was higher in the sensitization+challenge mice and their number further increased when stimulation protocol was performed suggesting more susceptibility of these cells to release IL-17 when both sensitization and challenges are performed.

Gene analysis in the ear tissues

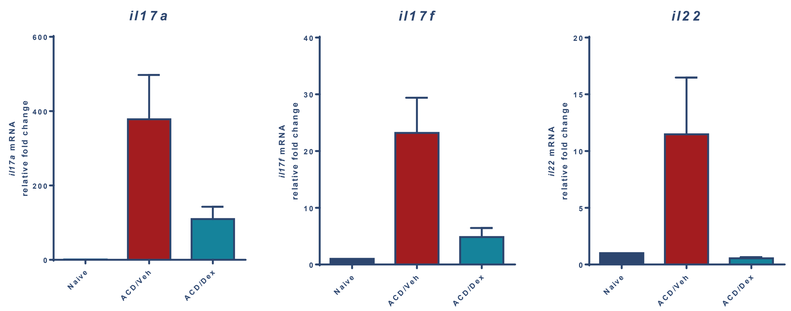

Figure 3: Gene analysis for il17a, il17f and il22 genes were performed in ear tissues of mice terminated on Day 14 of the model. Results show a clear induction of il17 and il22 genes in the urushiol ACD ear tissues which are known to be released from Th17 and γδ T cells in various dermal inflammatory conditions. Similar to the in-life results, treatment of the ACD mice with steroidal drug dexamethasone reduced the expression of these genes in the ear tissues.

4 CONCLUSION

In conclusion, we have established a protocol for induction of an allergic contact dermatitis model in mice using the poison ivy active ingredient, urushiol. Our results also showed infiltration of Th-17 cells in the ACD site in mice. Moreover, our results showed that Th17-derived cytokines might be involved in the urushiol-induced ACD.

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