Role of TSLP in experimental mouse models of type 2 inflammatory diseases

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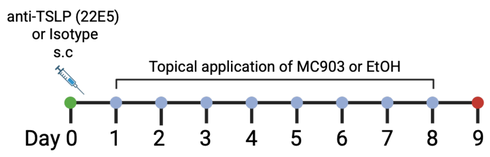
Introduction

- Atopic dermatitis (AD) and eosinophilic esophagitis (EoE) are inflammatory diseases of the epithelia driven by type 2 inflammation[1]
- EoE shares immunologic pathways, susceptibility loci and clinical features with AD[1]
- Thymic stromal lymphopoietin (TSLP), a master regulator of type 2 inflammation, is an alarmin secreted by barrier tissue epithelial cells in response to danger signals, infectious agents, food allergens and environmental antigens[2]
  - Single nucleotide polymorphisms in TSLP are linked to Th2 driven diseases, including EoE[3]
- TSLP inhibition has proven to be a clinically effective therapeutic option for reducing exacerbations and symptom burden in asthma patients with type 2 and non-type 2 inflammation[4]
  - TSLP inhibition is being explored as a potential therapeutic option for patients with other type 2 driven inflammatory diseases such as EoE

Methods

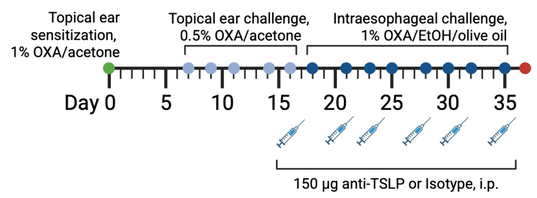
- All experiments were approved by local Animal Care Committees and performed in accordance with regulations and guidelines regarding the care and use of animals for experimental procedures

Figure 1: Experimental AD induction in Balb/c and TSLPR$^{-/-}$ mice



- Ear thickness was measured at baseline and day 9
- Histology scoring was performed by blinded pathologist assessment of H&E stained FFPE sections
- mRNA expression was performed by RT-qPCR analysis of mouse ears
- Serum protein expression of TARC was assessed by ELISA, and other cytokines/chemokines were assessed by Luminex

Figure 2: Experimental EoE induction in C57BL/6 mice



- Esophageal TSLP was measured by ELISA Days 0, 17, 19, 24, 29 and 36.
- Esophageal eosinophil counts, epithelial and lamina propria thickening, epithelial cell proliferation and vascularization were assessed by IHC on day 36.
- Statistics were performed as one-way ANOVA, unpaired 2-tailed Student's T test with 95% confidence intervals. Data plotted as mean ± SEM.

Conclusions

- TSLP is a pathogenic driver of disease in mouse models of AD and EoE
- TSLP inhibition reduced inflammation in the skin in the MC903 mouse model of AD
  - Cytokine inhibition by anti-TSLP analog (22E5) reduces cytokine expression to similar levels as in TSLPR deficient mice
- TSLP inhibition reduced eosinophil accumulation, epithelial and lamina propria thickening, and basal cell hyperplasia in a mouse model of EoE
- There is a complex interplay between IL-4, IL-13 and Th2 cells. TSLP inhibition has potential to reduce disease in type 2 inflammation driven epithelial diseases. Further clinical studies are required to demonstrate efficacy of TSLP inhibition in these two conditions.

Results

TSLP inhibition reduces inflammation in a mouse model of atopic dermatitis

Figure 3: Prophylactic administration of 22E5 and TSLPR KO reduces (**A**) ear thickening and (**B**) histologic scores compared to isotype controls. (**C**) Representative images of H&E stained ear sections. n=2-10

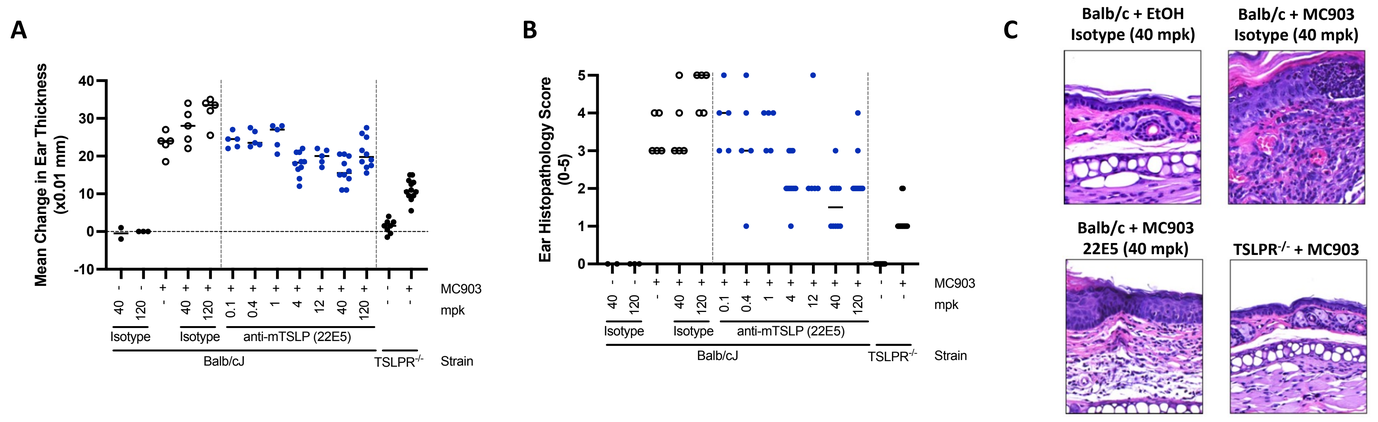
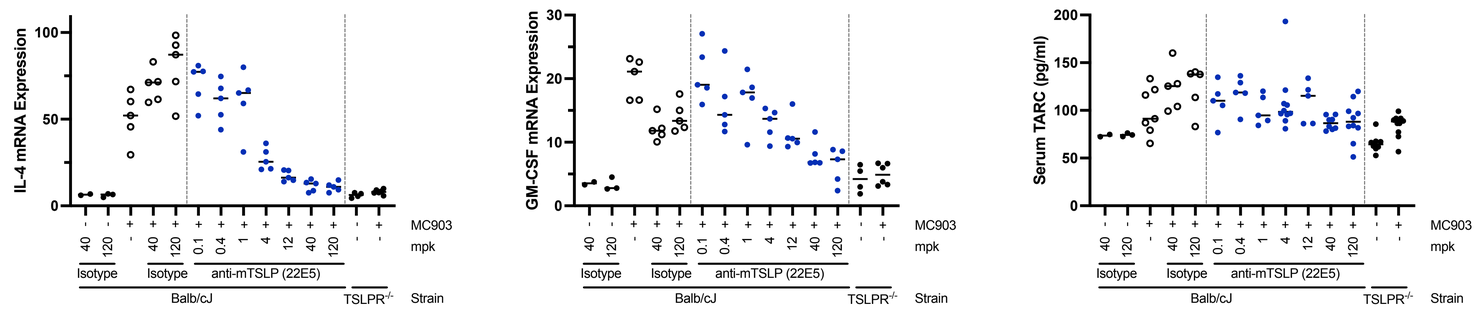


Figure 4: Prophylactic administration of 22E5 and TSLPR KO reduces tissue mRNA expression of (**A**) IL-4 and (**B**) GM-CSF, and (**C**) serum protein levels of TARC compared to isotype controls. n=2-6



- 22E5 also demonstrated a dose responsive reduction in IL-6, MMP12, and CCL3 mRNA expression, with the highest doses reducing to levels similar to the TSLP$^{-/-}$ mice (data not shown)

TSLP inhibition attenuates inflammation in a mouse model of eosinophilic esophagitis

Figure 5. TSLP regulates eosinophil infiltration into the esophagus

(**A**) Representative images of FFPE esophageal tissue stained with anti-MBP. (**B**) Quantitation of MBP+ cells in FFPE tissue. n=8-9, * p<0.05,*** p<0.001, **** p<0.0001.

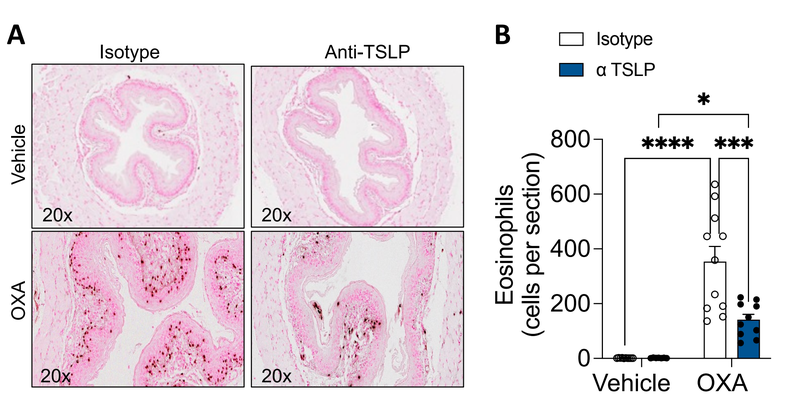
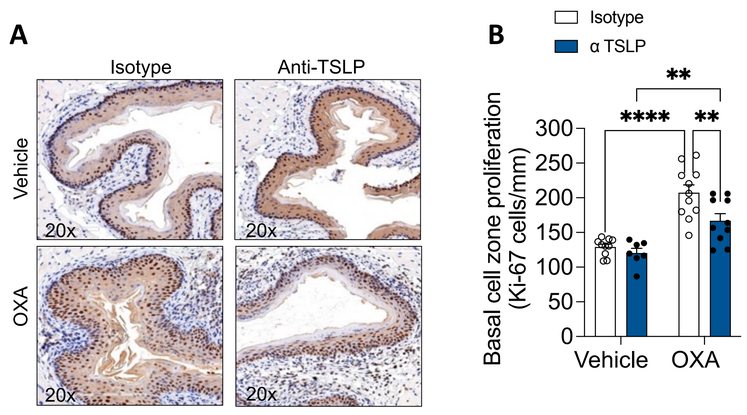


Figure 6. TSLP neutralization reduces epithelial basal cell hyperplasia

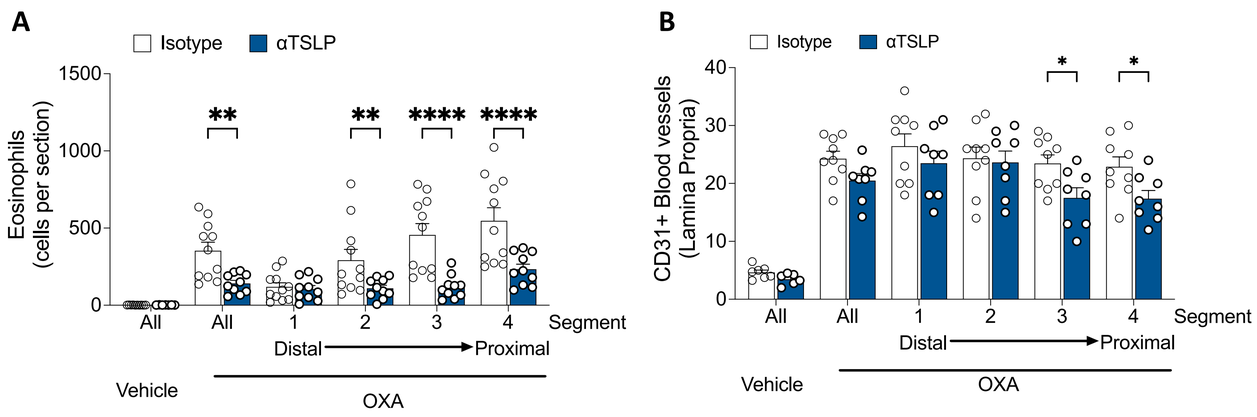
(**A**) Representative images of FFPE esophageal tissue stained with Ki-67. (**B**) Quantitation of Ki-67+ cells in FFPE tissue. n=8-9, ** p<0.01, **** p<0.0001.



- 22E5 treated mice reduced lamina propria thickness (62.2 mM) compared to isotype treatment (73.1 mM). p<0.05

Figure 7. TSLP neutralization reduces vascularization in the esophageal segments with the greatest eosinophil infiltration

(A) Eosinophil infiltration by esophageal segment. (B) CD31 staining by esophageal segment. n=6-11, * p<0.05 ** p<0.01, **** p<0.0001.



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Disclosure of interest
HKK, LW & HO are employees and shareholders of Uniquity Bio